UNIVERSITY OF EDINBURGH

A Thesis submitted

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a candidate to qualify

for the degree

of

Doctor of Philosophy

May, 1940

Title.

Studies on Seed Mucilages.

CONTENTS

GENERAL INTRODUCTION

<1> Although a vast amount of research has been done in the field of carbohydrate chemistry, comparatively little attention has been given to the group of substances which may be classified under the heading of acid polysaccharides. In this group we find such substances as pectins, plant gums, hemicelluloses, mucilages, soluble specific substances produced by bacteria, and alginic acid from algae. Most of these substances appear to be formed by the modification of cellulose under the influence of enzymes, few, if any of them, being produced directly by photosynthesis. Acid polysaccharides apparently play a very important, though poorly understood, role in natural processes.

Among the members of this class of substances, which are proving to be of great interest in modern research, are the plant gums and mucilages.<3> Under the name of gums a variety of substances have been described which differ considerably from one another both chemically and physiologically. The plant gums and mucilages, as is well known, are salts of very complex organic acids, usually with calcium, magnesium and potassium. These complex acids are built/

built up of hexose, pentose and methylpentose units in combination with the acid part of the molecule. The facts that many of them liberate carbon dioxide on treatment with boiling 12% hydrochloric acid, and that they give the naphtho-resorcin test (1), indicate that they contain uronic acid units.

Most of the early workers in this field were quite unaware of the occurrence of an acid portion in the molecule and treated the polysaccharide as if it were simply a polyose. This is quite excusable for the times, as the percentage of uronic acid is never very high and the method for estimating it had not been worked out. It was only from the papers of O'Sullivan that it was realized that the skeleton of the molecule was an acidic portion. His pioneer work, the investigation of gum arabic (2), geddah gum (3), and gum tragacanth (4), is remarkable for the difficulties overcome, since at that time (1880-1900) not all the simple sugars had been described or their constants determined. Briefly, his methods were partial hydrolysis with acid, preparation and fractional separation of the barium salt from the hydrolysis liquid, and then the ultimate analysis of the salt. In this gum arabic work, he hydrolysed arabic acid with dilute

sulphuric/

sulphuric acid and isolated a stable acid of lower molecular weight which he called γ-arabinosic acid. By analysis he assigned to this substance the formula $C_{23}H_{38}O_{22}$. He also claimed in his other papers to have isolated similar C_{23} acids from gum tragacanth and geddah gum. A few years later the work of Robinson (5) on the gum obtained from the shrub Cochlospermum gossypium backed up these findings of O'Sullivan. Robinson also claimed to have proved the occurrence of a C_{23} acid. As a matter of fact, until quite recently, it had been accepted, on the basis of O'Sullivan's work, that the acidic nucleus of gums is a complex organic molecule of unknown constitution, containing 23 carbon atoms in combination with hydrogen and oxygen as indicated above. During the last decade there has been a decided re-awakening of interest in the chemistry of acid polysaccharides, and many new and interesting facts have been discovered. The recent advances in this branch of carbohydrate chemistry have, therefore, rendered O'Sullivan's conclusions invalid but to him is due the discovery that the general pattern of the plant gums, and also mucilages, is that of a complex acid nucleus to which is/

is attached sugar units which are readily hydrolysed. The acidity of the carbohydrates studied has been shown to be due to hexuronic acids. Three of these aldehyde sugar acids have been discovered to date, namely d-glycuronic acid, d-galacturonic acid and d-mannuronic acid, corresponding in configuration to the three commonly occurring hexoses. Apparently the uronic acids are not found free in plants, but occur in highly complex molecules, sometimes as polymers of the uronic acid itself as in algin, and sometimes in combination with pentose, methyl pentose, and hexose sugars.

The really intensive study of gums and mucilages did not commence till the nineteen twenties however, but between this time and the time of O' Sullivan's pioneer efforts a very similar type of polysaccharide was occupying the minds of research chemists. This was pectin. A brief consideration of the work on this field will show the trend and gradual advance of polyuronide chemistry.

Most chemists nowadays, know that pectic substances are a group of polyuronides. From the botanical point of view they are of considerable importance, inasmuch as the young cell wall of meristematic/

meristematic tissue, and later, the middle lamella of older tissues is believed to be pectic in nature. Fruits of all types contain considerable quantities of pectic substances, the presence of which, later, confers upon jams and preserves their typical jellying properties. These substances are apparently present in all plant tissues with the possible exception of woods, though in some cases the amounts are very small. The state of knowledge of the structure of pectin at the beginning of the twentieth century was simply that the basal unit was pectic acid (which has since been shown to be predominantly a polygalacturonic acid, although containing in addition some sugar unit or units). This pectic acid was known not to occur in the free state but as the insoluble calcium or calcium-magnesium salt, particularly in the middle lamella of plants.

The modern period of research began with the work of von Fellenberg. He demonstrated thoroughly the presence of arabinose and galactose and showed that mild treatment with alkali splits off methoxyl groups with the formation of the calcium salt of pectic acid. He therefore regarded the water/

water soluble pectin as a methyl ester of pectic acid (6). Notice that up till now the acid part had not been characterised. This task was left to F. Ehrlich whose brilliant researches were taken up in 1917 and are still continuing. In 1917 both Ehrlich (7) and Saurez (8) discovered the hitherto unknown d-galacturonic acid in the hydrolysis liquid of lemon pectin and Ehrlich proved that the acidity of pectin was due entirely to galacturonic acid units which constitute the greater part of the molecule. In his various papers Ehrlich came to the conclusion that pectin could be formulated in a general way as a calcium-magnesium salt of an acid consisting of a methoxylated galactose-tetra galacturonic acid complex, to which arabinose is loosely attached, in some samples arabinose being replaced by methyl pentose. Later work by von Fellenberg (9) confirmed the conclusions of Ehrlich as regards the constituents but naturally von Fellenberg had his own ideas of the structure, considering arabinose as an integral part of the molecule. The real advance in the work however was brought about by the researches of Nanji, Paton and Ling (10) who put the work on a strictly quantitative basis. They advanced accurate values for the uronic content and pointed out that the uronic acid yielded also some furfural when treated with 12% acid. This

amount/

amount had to be taken into account when estimating the pentoses. As a result of their work they advanced a possible basal formula composed of tetragalacturonic acid and one unit each of arabinose and galactose, arranged in a ring. The idea has been widely accepted but recently it has been shown (11) that the connection for furfural from the uronic acid was incorrectly based. Their formula, then, rather loses its significance. It is only in very recent years, however, that the work of Link (12) has proved the polygalacturonic acid to have a chain structure, which is justified by the X-ray observations and viscosity measurements. Link's work dealt with the formation of polygalacturonic acid-methyl glycosides and the subsequent isolation of α-methyl d-galacturonide-methyl ester. Even so the present position of our knowledge of the structure of pectin is still far from satisfactory and indeed chemists have not decided whether pectins from all sources are predominantly the same or not. It is also generally believed that the sugars galactose and arabinose are not integral parts of the molecules but are derived from the arabans and galactosans in the cell wall. However, all the above research work certainly lays stress on the point of interest to workers/

workers in similar fields, namely, the uronic acid part of the molecule.

Returning now to study the gums and mucilages again it is noticed that the subject really began to take shape about 1926 when Heidelberger and Goebel (13) grew pneumococcus in a solution containing glucose, whence a polysaccharide was formed. They analysed this polysaccharide and showed the occurrence of a uronic acid residue. They of course did not concern themselves with linkages but were able to say that this product was a glucose-glucuronide. On mild hydrolysis they were amazed to find no free glucose but instead a product which analysis proved to be a disaccharide. This product was composed of 50% hexose and 50% uronic acid and was the stable skeleton of the polysaccharide. An aldobionic acid such as this has been proved to be the nucleus of all seed mucilages and also of a great many gums. Soon after this work was published papers dealing with gums and mucilages began to pour forth and gum arabic in particular claimed much attention and only quite recently has anything like a satisfactory structure been allotted to the molecule. Before going on to give a detailed discussion of the gums and mucilages I might mention that when I talk of/

of a gum I mean an exudation on the bark of a tree and I assume a mucilage to be an extraction from plant material, e.g. seeds. Also gums may be water soluble or water insoluble.

Now the most important and best known water soluble gum is gum arabic which finds many uses commercially as an adhesive. It cannot be assumed, till conclusive evidence is put forward, that gum arabic is, from different species, identical in composition, though perhaps constructed in the same general way. The gum appears in nature as a salt. Information on the structure of gums and mucilages is most readily gained by regulated hydrolysis, identification of the units removed and examination of the residual acidic body. On extensive hydrolysis of gum arabic, the residual acidic body was found to be an aldobionic acid (14), later shown to be constructed of galactose and glucuronic acid units. This was a most interesting discovery since such acids had only previously been found in certain bacterial polysaccharides with specific immunological properties. On the basis of various common reactions the structure of the aldobionic acid was presumed to be α (or β) -glucurono-3(or 6)-α-galactose (15). With the introduction of methylation, the research done in recent years points, however, to the linkage occurring between/

between the reducing group of the uronic acid and carbon atom six of the galactose (16). Butler and Cretcher in their paper (17) presumed that the structure of the gum was a chain but the aldobionic acid alone was not the skeleton, the nucleus being a more complex grouping of which the aldobionic acid of course formed an important part. Norman (18) however thinks that the structure is much more complex than this and postulates the close association of the three galactose units and the uronic acid unit, giving a ring structure to which the arabinose and, if present, the rhamnose, is attached. It is only within the last few months that the structure of gum arabic has become more or less proved. This has been made possible of course nowadays because of the great advance in technique and by the discovery of really accurate methods for quantitative estimations. F. Smith (19) has published four excellent papers on gum arabic within the last year and has finally offered a tentative structure for the molecule agreeing with the quantitative and qualitative findings. He concentrated mostly on the degraded arabic acid, i.e. the gum with the arabinose units removed. By fully methylating this degraded product, and by subsequent hydrolysis with 6.5% methyl-alcoholic hydrogen chloride, he isolated the barium salt of

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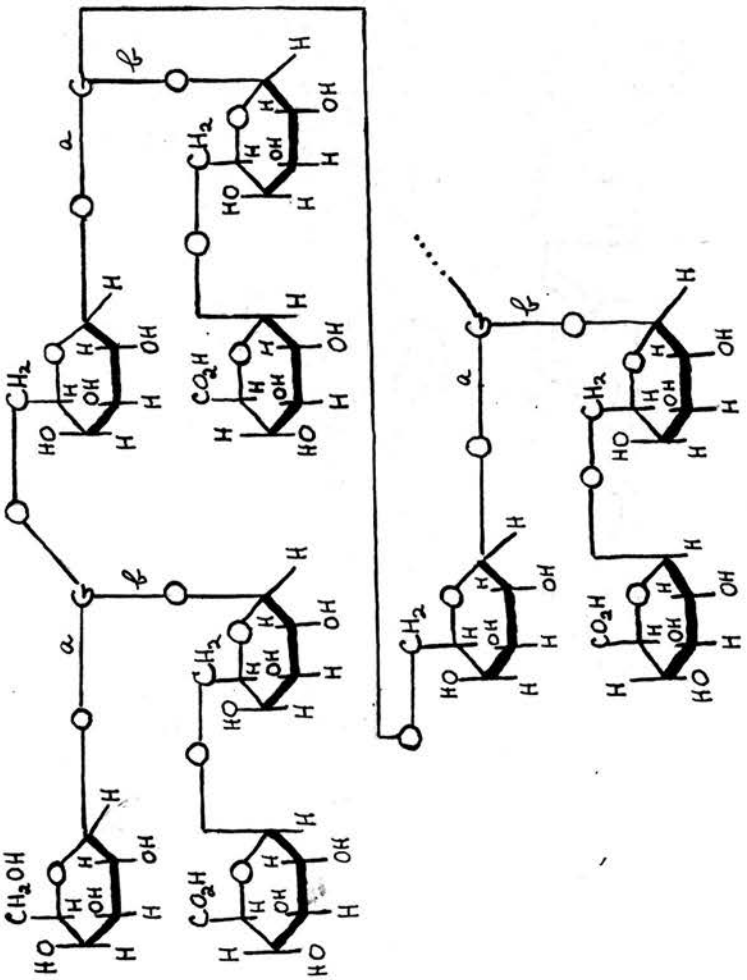
2:3:4-trimethyl methyl glucuronoside, 2:3:4:6-tetramethyl methyl galactoside, 2:3:4-trimethyl methyl galactoside and the 2:4-dimethyl methyl galactosides (α and β), whereas with 14 N sulphuric acid in the cold he isolated an aldobionic acid —— hexamethyl 6-β -glucuronosido-galactose. This aldobionic acid is hydrolysed with 3% methyl-alcoholic hydrogen chloride to yield 2:3:4-trimethyl methyl galacto-pyranose and 2:3:4-trimethyl methyl glucuronoside. The identification of 2:4-dimethyl and 2:3:4-trimethyl galactose shows that both the 1:6 and the 1:3 types of linkages are present in degraded arabic acid. Some of the galactose units are mutually joined by 1:6 links and the formation of 3-galactosido-galactose by prolonged autohydrolysis of the degraded arabic acid also points to the fact that some of the galactose units are joined by 1:3 links. From all this data Smith seems quite justified in offering the following structure, which is simply a chain of galactose residues to which are attached three side chains, each of which is terminated by a residue of glucuronic acid:-

There remains to be determined the positions of the glycosidic links represented by "a" and "b" in the figure.

Tentative Structure for Arabic Acid (F.Smith).



G = galactose unit.

Quite a few other gums have attracted attention from time to time but none have been studied on such a scale as gum arabic. In fact none of these have been given very accurate structures at all, the constituent parts being simply characterised.

Water soluble mesquite gum is found as droplets exuding from the stem and branches of the mesquite tree, Prosopis juliflora. On hydrolysis, arabinose, galactose and glucuronic acid were thought to be present (20). Also more detailed work showed a remarkable resemblance in general structure between mesquite gum and gum arabic (21). Analyses of the gum acid agree closely with a molecule composed of four units of l-arabinose, three of d-galactose and one of a methoxy-d-glucuronic acid. According to the nature of the hydrolysis the uronic acid was found to be joined to three, two, and one molecule of galactose. In the latter case an aldobionic acid was obtained and consists of galactose and methoxy-glucuronic acid. The fact that even under extremely strong conditions the methoxyl group could not be removed, points to its being on position 2, 3 or 4 (not 1 or 6).

Several gums do occur however which are mixtures of water soluble and water insoluble fractions, the latter/

latter usually predominating. The chief of these is perhaps gum tragacanth, from various species of Astralagus, found mostly in Greece and Turkey. On addition of water the gum swells to give a thick, viscid, mucilaginous liquid, about 60% of the gum not passing into solution but forming instead a bulky jelly. This insoluble fraction has been termed "bassorin" but has never been fully examined. The soluble fraction, tragacanthin, is filtered off and precipitated with alcohol. This fraction has been found to have a very high uronic content —— in the region of 51% (22). On hydrolysis the only sugar that could be detected was arabinose and finally a resistant nucleus was isolated which analysis proved to be made up of four uronic acid units and one arabinose unit. The high uronic acid content is most interesting and there is a possibility of the occurrence of a tetrauronic portion as in pectin. The work done on this gum however, is very sketchy. Two types of formulae are possible of course, as in the case of pectin, a chain and a ring structure.

A similar gum to that above is cholla gum, from the white cholla cactus, Opuntia fulgida, of south western U.S.A. Two fractions are again possible and different proportions of the following units occur/

occur in each, galacturonic acid, rhamnose, galactose and arabinose (23). According to the conditions different sizes of acidic molecules could be isolated as above and this points to the probability of a chain structure. As above this gum has not really been fully examined.

Finally there is a gum which is practically insoluble in water, namely cherry gum, exuded from wild cherry trees. The gum is similar in pattern to the other gums but it is much more complicated owing to the large number of sugars present. Arabinose, xylose, galactose, mannose and glucuronic acid have all been identified in the hydrolysis liquid (24). This gum has been taken up as a research subject quite recently and the new work will be described later when modern researches are described.

And now, as this thesis is on the mucilage from rib grass seed, it is time to consider in detail the history of the work on mucilages. As far back as 1913 Neville (25) had realised that the mucilage from flaxseed yielded sugars on hydrolysis. He also thought it possible that the mucilage was a salt of a complex acid akin to the gums. In his work however he characterised no acid portion/

portion but postulated the occurrence of the sugars glucose, galactose, arabinose and xylose in the hydrolysis liquid. No other reference to work on plant mucilages can be found from that time till 1930 when Anderson and his co-workers began to tackle the subject systematically. Funnily enough flaxseed mucilage was the one that claimed attention at first. Anderson and Crowder (26) treated the mucilage with boiling 4% sulphuric acid and isolated an aldobionic acid which analysis showed to consist of galacturonic acid and l-rhamnose. Among the hydrolysis products they found only l-galactose and d-xylose and proved that glucose and arabinose were definitely absent. The really interesting part of this work lay in the isolation of l-galactose not d-galactose which is the form that usually occurs in nature. Furthermore as galacturonic acid was the uronic acid, it looked very much as if the laevo form of this acid was also going to be discovered. Research however showed definitely that the acid was dextro-rotatory. There is therefore in flaxseed mucilage the very unusual association of a laevo-rotatory sugar and the corresponding sugar acid of opposite optical form.

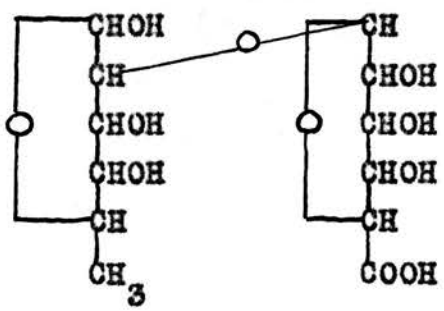
Linseed mucilage was expected to have a very similar/

similar constitution but unfortunately very little has been published regarding its structure. Norris and Marshall have examined it but only assert the occurrence of arabinose, galactose, rhamnose and galacturonic acid. They also mention that there is cellulose in combination with the polysaccharide and methoxyl groups have been proved to be present. It is noteworthy that cellulose is a very common substance extracted along with these mucilages. Exactly how it comes to be present and how it becomes soluble is not quite understood yet. It is found in quite a large amount in quince seed mucilage; Renfrew and Cretcher (27) quote that after hydrolysis of the mucilage there is a bulky residue amounting to about one-third of the weight of the dry mucilage. This mucilage is extracted from the seeds of the Persian quince, Cydonia vulgaris, in the form of a water soluble salt but queerly enough when it is precipitated from an acidified solution, the resulting free acid mucilage is insoluble in water. Analysis of the mucilage showed the occurrence of methoxyl groups. As usual hydrolysis brought about the formation of aldobionic acids which a Zeisel determination proved to be composed of 72% of a mono-methylated aldobionic acid mixed with about 28% of a /

a similar but unmethylated acid. The hydrolysis liquid yielded arabinose. The above aldobionic acids proved to be very resistant to acid hydrolysis with the result that the uronic acid has never been characterised, but xylose has been given as the unit associated with it. Renfrew and Cretcher examined the cellulose portion and asserted that 28% of it was soluble in 17.5% sodium hydroxide and this proportion therefore can be regarded as γ-and β-cellulose. The residue, α-cellulose, on hydrolysis by the Monier-Williams method, yielded 78% of crystalline glucose.

Mucilages, however, are not all obtained from seeds, as Anderson demonstrated in his work on the unusual mucilage obtained from the inner bark of the slippery elm, Ulmus fulva (28). This mucilage can be extracted in exactly the same way as in the case of seeds and also occurs as a salt. The free acid however, unlike that from quince seeds, is readily soluble in water, as are usually all free acid mucilages. Anderson, as of course did all the chemists at this time, merely characterised the units present. On hydrolysis he isolated an aldobionic acid consisting of l-rhamnose and d-galacturonic acid and in the hydrolysis liquid proved the presence of galactose. It is noteworthy that rhamnose-galacturonic acid should/

should have been identified in mucilages of such dissimilar botanical origin as the bark of the elm and the seed of the flax. The study of this unusual mucilage has been taken up again quite recently by Gill, Hirst and Jones at Bristol (29). They were of course more interested in how the units were linked up and indeed this is part of the work the modern chemists are stressing more and more, so that substances can be given a definite structure. The above workers concentrated on the aldobionic portion which they obtained by hydrolysis of the mucilage with normal sulphuric acid. They methylated this portion fully by the thallium method, followed by several Purdie methylations and hydrolysed it with 2N hydrochloric acid. From the hydrolysis liquid they isolated 2:3:4-trimethyl d-galacturonic acid and 3:4-dimethyl rhamnose. From these results it follows that the aldobionic acid is 2-d-galacturonido-l-rhamnose and is identical with the aldobionic acid which Tipson, Christman and Levene have isolated from flaxseed mucilage (30).



Anderson's latest contribution appeared in 1935, in a paper on Psyllium seed, Plantago psyllium (31). This seed yields as much as 20% of mucilage but this unfortunately is not at all homogeneous. The authors showed that the product varied quite considerably according to the amount of water used and the force used in the filtration process. Generally speaking the most easily soluble portions were those of higher uronic acid content. So that if only a little water was used in the extraction then the product had a higher uronic content and lower pentosan content than if a large amount of water was used. As usual the units were simply characterised as d-xylose, l-arabinose and d-galacturonic acid, the xylose being the more loosely bound. The aldobionic acid was therefore an arabinose-galacturonic acid.

From the above descriptions it is apparent that seed mucilages are definitely not homogeneous. Bailey realised this and devised an unusual but ingenious method for partial fractionation (32). A description of this method is given in his paper on the mucilage from white mustard seed, Brassica alba. By treatment with saturated baryta the aqueous extract could be divided into three fractions, two of which were polyuronides and the third cellulose. From one of

the polyuronide fractions, in the usual way, were isolated arabinose, galactose, and an aldobionic acid consisting of l-rhamnose and d-galacturonic acid. Galactose was the chief unit present and accounted for about half of the molecule. The other polyuronide fraction also consisted largely of galactose with some arabinose but in addition to d-galacturonic acid, d-glucuronic acid was also postulated to be present. A few years later Bailey examined cress seed, Lepidium sativum, along similar lines and found that the same procedure held good (33). It is interesting to note that in this case the mucilaginous layer of the seed swells but does not disperse and can only be separated by vigorous shaking. The structure of this mucilage appears to be quite complicated as on hydrolysis, apart from some arabinose and galactose, a complex salt is obtained which on further hydrolysis yields a mixture of two aldobionic acids. Bailey expresses the opinion that the complex salt is composed of two aldobionic acids linked together and that the acids are a rhamnose-galacturonic acid and a galactose-galacturonic acid. It is natural for the times of course that some of the papers discussed are a trifle indefinite as the authors had not nearly the same facilities at ~~their~~ hand/

hand as the modern chemist has. The extent of the recent advances will be seen to a marked extent when the more modern work on this subject is described later.

Before discussing the results published during the last two years it would be well worth while giving a thought to the most remarkable polyuronide of all, namely alginic acid. This substance is of course, not a mucilage or a gum but is included under the heading of gel-forming substances. Marine algae contain two types of polysaccharides. One is soluble in cold water or dilute acid and is called fucoidin or fucosan. The other is extracted with sodium carbonate and is precipitated by the addition of acid from which it separates in a highly gelatinous condition. This latter product is algin and is a polyuronide. Analysis showed it to contain a very high percentage of uronic acid. In fact a great deal of work has been done on this polysaccharide and only recently a paper appeared from the Bristol Laboratories clearing up a great deal of the existing muddle. The early workers were most speculative but most of their conclusions have since been disproved.

As far back as 1915 Hoagland and Lieb postulated the appearance of d-xylose on hydrolysis (34). It

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was not till much later that two papers appeared claiming to have proved the uronic acid to be glucuronic acid (35) and (36). The authors of the second paper gave as additional proof the isolation of the cinchonine salt of the uronic acid. However it was not till Cretcher and Nelson took up the subject that anything like sensible results appeared. They paid more attention to the quantitative estimation of the uronic acid and showed that the polysaccharide was composed practically completely of uronic acid, explaining the occurrence of any xylose as due to the decarboxylation of the acid (37). Now every worker in this field has found that it is extremely difficult to hydrolyse the polyuronide. Nelson and Cretcher allowed algin to stand in 80% sulphuric acid at room temperature for about a week and found that extensive hydrolysis had taken place although about 20% of unchanged material was still left. They isolated the cinchonine salt of a uronic acid with a melting point different to that of either galacturonic or glucuronic acid. By oxidation of this uronic acid they obtained a dibasic acid whose amide and diphenyl hydrazide agreed very well with those for d-mannosaccharic acid. Their obvious conclusion was therefore that algin was mainly a polyuronide of/

of d-mannuronic acid (38). Independently mannuronic acid was postulated to be the uronic acid by Bird and Haas (39) and also by Miwa (40). It was noticed however, that the authors of all those papers quoted different properties for the cinchonine salts but this was shown by Bird and Haas (39) and later by Nelson and Cretcher (41) to be due to differences in degree of hydrolysis only. Nevertheless there was still great inconsistency in the figures given for the equivalent weight of the polyuronide, which should be 176 from theory. Dillon and McGuinness (42) however titrated the wet gel and gave 198 as their equivalent, mentioning that in all probability dry alginic acid is a lactone and that the polymerising unit is not the uronic anhydride but the complete mannuronic group itself. This view was later supported when Barry, Dillon and O'Muineachain claimed to have acetylated the polysaccharide, the acetyl figures agreeing closely with the above theory (43). However, as more modern research has shown it is very doubtful if it is really possible to acetylate alginic acid without degrading it. This was proved in a paper which was published only last December (44). The authors have concentrated on a degraded alginic acid which they obtained by treating the polyuronide with methyl-alcoholic/

alcoholic hydrogen chloride. This degraded compound was more amenable to chemical treatment and could be methylated fairly easily by the thallium method. The fully methylated algin was however still very stable towards hydrolysing agents and the authors found it necessary to use 50% nitric acid when it was simultaneously oxidised and hydrolysed to give i-dimethoxy-succinic acid, recognised as its crystalline dimethyl ester and amide. Really strong treatment with 4% methyl-alcoholic hydrogen chloride at 150° for twenty four hours however, did break down the molecule with the formation of 2:3-dimethyl methyl mannuronide. The constitution of this product was proved conclusively by its oxidation with bromine and then periodic acid to give glyoxylic acid and the half aldehyde of i-dimethoxy succinic acid. From these results it was now obvious that the units of mannuronic acid could be joined to one another either by 1:4 or 1:5 linkages. As the latter type of linkage has never been found before it is reasonable to state that alginic acid consists of units of d-mannuronic acid linked through carbon atoms one and four.

Throughout this introduction, the most recent results have been described at the appropriate places whenever possible but there still remain a few papers to be discussed which have appeared within the last two years. It will be fitting to describe them as

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a conclusion. Several schools of sugar chemistry are investigating these types of polysaccharides and some excellent work of this kind has appeared from Bristol. It is this work I am going to mention now.

In connection with his work on pectin, Hirst has taken up the study of numerous polyuronides, 1938 saw the appearance of the first paper and it dealt with damson gum (45). In it the authors were concerned mainly with the isolation of an aldobionic acid consisting of d-mannose and d-glycuronic acid although they also proved the presence of arabinose, galactose and some xylose. They showed that on auto-hydrolysis of the free acid gum arabinose was split off leaving a degraded molecule containing galactose and the aldobionic acid. Further hydrolysis using acid served to remove the galactose. The aldobionic acid was then fully methylated and hydrolysed with difficulty with methyl-alcoholic hydrogen chloride to give 2:3:4-trimethyl d-glycuronic acid and 3:4:6-trimethyl d-mannose. This proved that the structure of the aldobionic acid was a molecule of d-glycuronic acid linked through its aldehyde group to carbon atom two of the mannose residue. The completion of this work seemed near at hand when a second paper appeared in September 1939 dealing with the fully methylated degraded damson gum and its hydrolysis products (46).

products (46). In the hydrolysis liquid the following fractions were proved to be present; 2:3:4:6-tetramethyl d-galactose (1 part), 2:4:6-trimethyl d-galactose (1 part), 2:3:4-trimethyl d-galactose (1 part), 4:6-dimethyl d-galactose (1 part), 2:3:4-trimethyl d-glycuronic acid (1 part), 2:3-dimethyl d-glycuronic acid (1 part), and a small quantity of 2:3:4-trimethyl d-xylose (1/6 part), together with an unidentified methylated derivative of d-mannose. Obviously once the mannose portion and the arabinose portion have been identified a tentative structure will be possible.

Actually before the above second paper had appeared, the first part of another study had been published. This paper dealt with the structure of cherry gum (47), a problem which had been tackled many years ago by Butler and Cretcher. The similarity of cherry gum and damson gum is indicated by the identity of their products of hydrolysis and especially of the d-β-glycuronosido-2-d-mannose obtained on the hydrolysis of the arabinose free polysaccharide. Cherry gum was also found to contain only about 1.5% of xylose. In this paper use is made of the unusual thallium method of methylation which is mentioned in various places throughout this introduction./

introduction. It is most unfortunate that the existing crisis may delay the ultimate completion of this fascinating work.

From a broad survey of all the work cited in this introduction it would seem highly probable that the specific nature of each acidic carbohydrate had its origin in the chemical identity of its component acid nucleus. Unfortunately, as far as the studies have gone, the fact that botanically different gums and mucilages have yielded identical acidic nuclei, is substantial evidence against this idea. It still remains for the results of modern researches to show how these carbohydrates can be satisfactorily classified with regard to their chemical properties.

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INTRODUCTION.

In this thesis the structure of the mucilage from rib grass seed, Plantago lanceolata, is investigated. The study falls into two main sections, the first dealing with the products obtained from the free acid mucilage itself and the second with the products obtained from the fully methylated mucilage. It is interesting to note that the British Pharmaceutical Codex (1) asserts that Plantago lanceolata seed gives no mucilage with water. In practice, however, as will be shown later, a 4-5% yield of mucilage can be obtained. Now a great deal of confusion arose at one time regarding the exact species of the various Plantago seeds on the market. Plantain seeds are really of Indian origin and in the literature references can be found pointing to the occurrence of several species in native Indian commerce. They are sold in the bazaars under native names and one of the best known of these is "Bartung" or "Barhang". Considerable disagreement has existed as to the origin of this drug, and although Irvine attributed it to Plantago lanceolata (2) research showed that the parent species was Plantago major (3). Another substance which/

which finds extensive use in pharmacy in the East is "Ispaghula", which consists of the dried, ripe seeds of Plantago ovata, a herbaceous annual indigenous to India and Persia. Psyllium seeds were also regarded as the seeds of Plantago Psyllium but commercial samples are sometimes composed entirely or partly of seeds of Plantago arenaria or Plantago lanceolata. Recent research, published in the Quarterly Journal of Pharmacy and Pharmacology gives a quick method of differentiating between the various species of plantain seed (4). It is based on the weights and dimensions of the seeds and forms quite a good method of checking up on the seeds under observation.

Up to the present time the problem of the structure of seed mucilages has not been attacked by methylation methods although Hirst and his co-workers have recently published an account of the structure of a fully methylated aldobionic acid occurring in the mucilage from the inner bark of the slippery elm, Ulmus fulva (5). This aldobionic acid is identical with the aldobionic acid isolated from flaxseed mucilage by Tipson, Christman and Levene (6), and consists of d-galacturonic acid and l-rhamnose joined by a 1:2 linkage.

The/

The paper which was consulted in the early part of the work, i.e. the hydrolysis of the mucilage etc. was that of Anderson and Fireman (7), on the mucilage from the seeds of Plantago Psyllium. These authors considered that the mucilage was not homogeneous but that the uronic acid and pentosan contents varied with the amount of water used and the force employed in the filtration process. The use of a small volume of water for a short time and the application of little force in the filtration led to the production of small amounts of the mucilage with high ash content and high uronic acid content but low pentosan content. On the other hand the use of more water, longer extraction and greater pressure in filtration led to the production of larger amounts of mucilage with lower ash and lower uronic acid content but higher pentosan content. It thus appears that polyuronides with high uronic acid content dissolve most readily. Anderson and Fireman hydrolysed the mucilage with 4% sulphuric acid and separated the products into three main fractions namely an insoluble X-body (probably cellulose), the salt of a uronic acid-sugar compound and a syrup containing free sugars. According to the duration of the hydrolysis they found that the uronic acid was linked to one or two pentose units.

When/

When the hydrolysis was for 20 hours an l-arabinose-d-galacturonic acid was isolated but if the hydrolysis was only carried out for 12 hours a d-xylose-l-arabinose-d-galacturonic acid could be obtained. The calcium salt of these acids, on further hydrolysis with 4% sulphuric acid in an autoclave at 120°, gave barium d-galacturonate together with the free sugars xylose and arabinose. The relative proportions of the two pentoses, xylose and arabinose, in the mucilage, were not determined, the total pentosan content only being stated. The authors of course did not give a structure to the mucilage as they did not consider the subject in the light of methylation methods.

An attempt has been made in this thesis to show how the various units are linked to one another, by the hydrolysis of the fully methylated compound, and the subsequent identification of various partially methylated products.

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E X P E R I M E N T A L

Part I

The Study of the "free acid" Mucilage

PREPARATION OF THE MUCILAGE.

The seeds (500 g.), purchased from David Bell and Sons, Coburg Street, Leith, were soaked in water (10 l.) for 24-30 hours. The thick, sticky mass was stirred vigorously from time to time as the seeds tended to settle to form a hard cake. The seeds were finally separated from the mucilaginous solution by filtering through muslin, suction being applied to hasten the process. The mucilage was obtained by pouring the brownish filtrate (500 c.c.) into vigorously stirred alcohol (1 l.) from which it separated as a stringy mass. The whole of the mucilaginous solution was precipitated in small amounts in this manner. The product was dehydrated by standing in absolute alcohol (1 l.) and after pressing free from alcohol was steeped in ether (1 l.) pressed and dried in a vacuum desiccator over calcium chloride. Yield 25g. Ash by direct incineration 5%; as sulphate 7%. The ash was shown to be an integral part of the molecule by dialysing some of the mucilage in a parchment bag for a week whereupon no reduction of the ash content was observed. Qualitative tests showed the presence of sodium, potassium and calcium.

A quantitative analysis of the ash gave:-

Sodium /

Sodium,	estimated	as	sodium zinc uranyl acetate	2.3%
Potassium,	"	"	potassium perchlorate	14.5%
Calcium,	"	"	calcium oxalate	18.8%
Sulphate,	"	"	barium sulphate	53.2%

Preparation of the "free acid" Mucilage. (1)

The mucilaginous solution was prepared from the seeds as described above. The "free acid" mucilage was obtained by pouring the viscous extract (500 c.c.) into vigorously stirred acidified alcohol (1 l. containing 20 c.c. of concentrated hydrochloric acid) from which it separated as before in a fibrous condition. The whole of the extract was precipitated in small amounts in this manner. The product was dehydrated by standing in absolute alcohol (1 l.) and was finally triturated with alcohol until free of chloride ions. The product was dried as described above. It was obtained in the form of a white, fibrous non-reducing solid (25 g.) which swelled in water to give a thick, viscous opalescent solution.

Analysis of the "free acid" Mucilage.

(a) Rotation. It was very difficult to see through a solution of the "free acid" mucilage in water. However, on centrifuging the solution for an hour, it was possible to obtain a reading.

$$[\alpha]_D^{16^\circ} -60^\circ \text{ (c, 0.4).}$$

(b) /

(b) Equivalent Weight. A known weight of the polysaccharide (ca. 0.25 g.) was allowed to stand overnight in a known volume (ca. 20 c.c. N/20) of standard sodium hydroxide solution. The excess of alkali was found by titration with a standard acid using phenolphthalein as indicator. The values obtained from five experiments ranged from 1070 to 1150.

(c) Methoxyl. No OMe groups present.

(d) Uronic Acid. The method used was that of Dickson, Otterson and Link (2) except for the inclusion of an aniline trap (3) to keep back any furfural which might distil over. In a typical estimation 3.8% of carbon dioxide was obtained corresponding to 15.2% uronic anhydride or 16.7% of uronic acid.

(e) Pentosan and Methyl Pentosan. The method used was that described by Marshall and Norris (4). The amount of pentosan was calculated from the weight of furfural phloroglucide using the appropriate factors (5) and the methyl pentosan estimated by the method of Ellett and Tollens (6). The results were corrected for furfural derived from the uronic acid (7). In a typical estimation the "free acid" mucilage (0.1801g.) yielded a mixture of furfural and methylfurfural phloroglucides (0.1510 g.). The methylfurfural phloroglucide /

phloroglucide (0.0115g.) was removed with hot alcohol, as described by Ellett and Tollens (6), indicating the presence of 72% pentosan and 11% methyl pentosan.

Attempted Autohydrolysis of the "free acid" Mucilage.

I. The "free acid" mucilage (5g.) was heated on the boiling water bath for 24 hours with water (200 c.c.). An insoluble residue (0.33g.) was filtered off and the filtrate evaporated to 30 c.c. at 45°/20 m.m. This solution was added slowly with stirring to alcohol (1 l.). The white solid produced was filtered, washed with alcohol and ether and dried in a vacuum (2.95g.); equivalent weight 960, $[\alpha]_D^{17^\circ} - 62^\circ$ (c,1.0 in water), pentosan 70%, methyl pentosan 13%. The filtrate and washings on concentration gave a pale yellow syrup (0.8g.), $[\alpha]_D^{15^\circ} +20^\circ$ (c,1.0; equilibrium value in water). After a few weeks crystals appeared which had m.p. 140°.

II. The same quantities of material were used but the hydrolysis was continued for 3 days. An insoluble residue (0.32g.) a white solid (1.35g.), equivalent weight 830, $[\alpha]_D^{15^\circ} -41^\circ$ (c,1.0 in water), pentosan 65%, methyl pentosan 17% , and a syrup (2.2g) were obtained as before.

The syrup (0.4g) was heated at 95° with phenylhydrazine hydrochloride (0.3g), sodium acetate (0.5g.) and sodium /

sodium bisulphite (0.05 g.) in water (10 c.c.). A yellow crystalline osazone was obtained (0.3 g.), m.p. 160°, mixed m.p. with an authentic specimen of xylosazone 158°, $[\alpha]_D^{16°} -40°$ (C, 0.9 in alcohol).

Acid Hydrolysis of the "free acid"Mucilage.

The rate of hydrolysis of the polysaccharide was followed using various strengths of acids, 4% - sulphuric, 4%-hydrochloric and 3%-oxalic acid. In each case $[\alpha]_D^{15°}$ changed from -60° to 60° in 3 hours but it was found that very little furfural was formed using oxalic acid, whereas the other acids yielded solutions which gave a strong positive test with aniline acetate paper.

Oxalic acid hydrolysis.

The mucilage (16.2 g.) was heated on the boiling water bath for 20 hours with 3%-oxalic acid (250 c.c.). The insoluble residue (1.14 g.) was filtered off and the filtrate neutralised with calcium carbonate in the presence of charcoal. The mixture was kept at 100° for half an hour and the filtrate evaporated to 50 c.c. at 45°/15 m.m. This solution was poured slowly into well stirred alcohol to yield a calcium salt 'A' (5.4 g.) which was washed with alcohol and ether and dried in a vacuum desiccator over calcium chloride. The filtrate and washings on evaporation at 40°/15 m.m. gave/

gave a pale yellow syrup 'B' (7.8g).

Study of the calcium salt 'A'.

The calcium salt gave:-

$[\alpha]_D^{16°}$ + 82° (c,0.5 in water), ash as sulphate 17.1%, uronic acid 45%, methyl pentosan 35.8%, pentosan nil.

Fractionation of the calcium salt 'A'.

To the calcium salt (10g.), prepared as described above, in water (250 c.c.) alcohol was added slowly with stirring until a reasonable amount of solid I had separated (75 c.c. of alcohol). This was removed on the centrifuge and dried (0.93 g.). More alcohol (200 c.c.) was added to the clear solution to give fraction II (4.7g.). A further addition of alcohol (325 c.c.) gave fraction III (0.63g.). The remaining solution was evaporated to 25 c.c. at 40°/15 m.m. and poured into alcohol (300 c.c.) to give a final fraction IV (1.47g.).

Analysis of the fractions.

I. $[\alpha]_D^{17°}$ + 89° (c,0.5 in water), ash as sulphate 17.0% uronic acid 44.3%, methyl pentosan 34.2%, pentosan nil.

II. $[\alpha]_D^{17°}$ + 80° (c,1.0 in water), ash as sulphate 16.8% uronic acid 43.1%, methyl pentosan 34.3%, pentosan /

pentosan nil.

III. $[\alpha]_D^{17^\circ}$ +74° (C, 0.7 in water), ash as sulphate 16.5%, uronic acid 41.0%, methyl pentosan 37.0%, pentosan nil.

IV. $[\alpha]_D^{17^\circ}$ +70° (C, 0.5 in water), ash as sulphate 16.7%, uronic acid 41.0%, methyl pentosan 37.5%, pentosan nil.

Examination of the Syrup 'B.'

On standing for a few weeks this syrup began to crystallise. The product was shaken up with glacial acetic acid and filtered. The crystalline material was washed several times with glacial acetic acid and dried, m.p. 142°, mixed m.p. with an authentic specimen of d-xylose 140°. $[\alpha]_D^{17^\circ}$ + 80° ⟶ + 18.2° in 24 hours (C, 0.7 in water). The filtrate was condentrated to a syrup 'C', $[\alpha]_D^{19^\circ}$ +80° (C, 0.5 in water). This product failed to crystallise even on standing in the refrigerator for several months.

The above crystalline sugar (0.4 g.) was heated on the water bath at 95° with a solution containing phenylhydrazine hydrochloride (0.3 g.) sodium acetate (0.5 g.) and sodium bisulphite (0.05 g.) in water (10 c.c.). A yellow crystalline osazone (0.34 g.) was obtained in 30 minutes, m.p. 158°, mixed m.p. with an authentic specimen of xylosazone 158°. $[\alpha]_D^{18^\circ}$ -44° (C, 0.4 in alcohol). /

alcohol).

This result was confirmed by the isolation of the characteristic boat-shaped crystals of cadmium bromide — cadmium xylonate (8).

Examination of Syrup 'C'.

It was attempted to prepare arabinose diphenylhydrazone from the syrup, a control experiment being run at the same time.

I. Xylose (0.1 g.) and arabinose (0.01 g.) were refluxed for 30 minutes with diphenylhydrazine (0.15 g.) and aqueous alcohol (8 c.c.; 1:1). The solution was kept at -3° overnight and yielded a crystalline product (0.026 g.), m.p. 204°d.

II. Syrup 'C' (0.35 g.) was treated in exactly the same way with diphenylhydrazine (0.5 g.) and aqueous alcohol (20 c.c.). No solid product was obtained even on standing for several weeks.

Another portion of syrup 'C' (0.2 g.) was heated on the water bath at 60° with concentrated nitric acid (5 c.c.) until the volume was reduced to 1 c.c. (3-4 hours). Water (5 c.c.) was added and the solution allowed to stand. After 24 hours crystals began to appear. These were filtered off after 2 days (0.06 g.), m.p. 223°d, mixed m.p. with an authentic specimen of mucic acid 222°d.

Other Attempts to hydrolyse the "free acid" Mucilage.

I. Hydrolysis with 4%-sulphuric acid.

The polysaccharide (20 g.) was treated with 4%-sulphuric acid (250 c.c.) on the boiling water bath for 24 hours. The insoluble residue (1.11 g.) was filtered off and the filtrate neutralised with barium carbonate to give a barium salt I (4.75 g.), $[\alpha]_D^{17^\circ}$ +69° (C, 0.5 in water), ash as sulphate 28.2%, uronic acid 49.5%, methyl pentosan 14.2%, pentosan nil, and a syrup (11.5 g.), $[\alpha]_D^{18^\circ}$ +34° (C, 0.7 in water).

II. Hydrolysis with 7.5%-sulphuric acid.

The conditions of the hydrolysis were exactly the same as above. An insoluble residue (1.86 g.), a barium salt II (4.1 g.), $[\alpha]_D^{17^\circ}$ +33.6° (C, 0.6 in water), ash as sulphate 32.2%, uronic acid 57.7%, methyl pentosan 7%, pentosan nil, and a syrup (10.9 g.) $[\alpha]_D^{17^\circ}$ +28° (C, 0.8 in water) were again obtained.

III. Hydrolysis with 15%-sulphuric acid.

The conditions were exactly the same as before only 15 g. of the polysaccharide were used instead of 20 g. Three products were again isolated namely an insoluble residue (1.93 g.), a barium salt III (1.81 g.) $[\alpha]_D^{16^\circ}$ +22° (C, 0.6 in water), ash as sulphate 50.1%, uronic acid 61.69%, methyl pentosan nil, pentosan nil; and a syrup (6.94 g.) $[\alpha]_D^{18^\circ}$ +25° (C, 1.0 in water).

Investigation/

Investigation of Barium Salt III.

A portion of this salt (0.5 g.) was dissolved in water (5 c.c.) and the calculated amount of dilute sulphuric acid added with stirring. The precipitated barium sulphate was centrifuged off and the resulting clear solution concentrated to a syrup (0.3 g.) at 45°/15 mm., $[\alpha]_D^{16°}$ +48.8° (C, 1.0 in water).

A portion of this syrup (0.1 g.) was treated with 50% nitric acid (5 c.c.) on the steam bath for 2 hours when the volume was reduced to about 1 c.c. Water (5 c.c.) was added and the whole left aside. After a few hours crystals began to appear and were filtered off after 2 days. The product (0.07 g.) had m.p. 225°d., mixed m.p. with an authentic specimen of mucic acid 224°d.

Glycoside formation.

The above syrup (0.043 g.) was treated with 1% methyl-alcoholic hydrogen chloride (10 c.c.) at 15°, $[\alpha]_D^{16°}$ +44.8° (initial value) +42.5° (3 hours), +23.3° (18 hours), ± 0 (36 hours), -19.7° (48 hours), -37.7° (72 hours), -42.4° (84 hours; constant value).

Glycoside formation using d-galacturonic acid.

$[\alpha]_D^{17°}$ in 1% methyl-alcoholic hydrogen chloride (C, 0.59) + 49.6° (initial value), + 46.3° (3 hours), +24.7°/

+24.7° (12 hours), ±0 (24 hours), -31.3° (39 hours), -51.2° (48 hours; constant value).

Discussion of Results

When rib grass seed, Plantago lanceolata, was soaked in water it gave a mucilaginous solution. cf. (9). Precipitation in alcohol resulted in the isolation of a non-reducing polysaccharide which had a definite ash content, not reduced by dialysis (5% - direct ignition and 7% as sulphate), and which appeared to consist in the main of sodium, potassium and calcium sulphates. If, however, acidified alcohol was used in the precipitation process (10) a product was obtained which had no appreciable ash content and which gave an acid reaction to Congo Red paper. The equivalent weight of this "free acid" polysaccharide was determined by titration, the values from five experiments ranging from 1070 to 1150. This value can be checked from the ash content of the crude polysaccharide, a value of 7% ash (as sulphate), assuming the presence of potassium and calcium in equal proportions, requiring an equivalent weight of ca 1100. The uronic acid content was determined by the method of Dickson, Otterson and Link (2), the average value from five experiments being 16%. This value is in reasonable agreement with the equivalent weight as determined by titration/

titration and by consideration of the ash content, assuming all the carboxyl groups of the uronic acid residues to be free in the polysaccharide.

The polysaccharide was hydrolysed completely in 20 hours by heating with 3%-oxalic acid, leaving an insoluble "cellulosic residue" amounting to 7% of the dry mucilage. Such residues have been repeatedly noticed in the hydrolysis of gums and mucilages.(11). The filtrate on neutralisation with calcium carbonate was separated into two fractions, namely a calcium salt, insoluble in alcohol, which appeared to be homogeneous since on reprecipitation from aqueous solution four identical fractions were obtained, and an alcohol soluble fraction. The ash content of the calcium salt (17%) indicated the probability that it may have been an aldobionate and on further analysis it was found to contain a methyl pentose residue (45%). The soluble fraction crystallised almost completely to yield d-xylose. This was confirmed by the preparation of an osazone which gave physical constants in agreement with those for d-xylosazone; and the isolation of the characteristic boat-shaped crystals of cadmium bromide-cadmium xylonate (8).

After/

After the removal of the crystalline d-xylose a small amount of syrup remained which did not crystallise even on standing for many months. This syrup was tested for the two sugars which occur frequently in polysaccharides of this type, namely d-galactose and l-arabinose. Attempts to isolate the easily obtainable arabinose diphenylhydrazone were unsuccessful but oxidation with nitric acid gave a poor yield of mucic acid indicating the presence of a small quantity of galactose in the syrup.

Several attempts were made by hydrolysis with various strengths of sulphuric acid to isolate a simple uronic acid from the "free acid" mucilage. 15%-Sulphuric acid was found to be the most suitable and the product obtained contained no pentose or methyl pentose residues and the ash content (50% as sulphate) was in reasonable agreement with that which would be obtained from a substance of the formula $C_6H_9O_7.\frac{1}{2}Ba$. This product appeared to be barium d-galacturonate for the following reasons:-

(1) $[\alpha]_D^{16°}$ of the free uronic acid in water $+48.8°$

$[\alpha]_D^{16°}$ of d-galacturonic acid in water $+51.0°$

(2) The/

(2) The free uronic acid, which was not obtained crystalline, however, yielded mucic acid on oxidation with nitric acid.

(3) The rotation of the free uronic acid in 1% methyl-alcoholic hydrogen chloride at room temperature changed from a strongly positive value to a strongly negative value in a few hours, a procedure characteristic of galactose derivatives having a free hydroxyl group on position C_4.

These preliminary experiments show therefore that the mucilage on hydrolysis yields chiefly d-xylose and this sugar probably comprises the entire pentose content (70%) since arabinose is absent. Apart from that the uronic acid (16%) appears to be d-galacturonic acid and a small quantity of galactose has also been identified. The identity of the methyl pentose associated with the uronic acid has not been ascertained.

Summary

1. On extraction of rib grass seeds with water and precipitation with alcohol a polysaccharide has been isolated with an ash content of 7% (as sulphate). Reprecipitation in alcohol containing hydrochloric acid yielded a polysaccharide which was almost free from ash.

2. This "free acid" mucilage gave the following results on analysis:- Acid Equivalent 1100, uronic anhydride 15%, pentosan 70%, methyl pentosan 11%.

3. Hydrolysis with oxalic acid yielded an insoluble residue (7%), the calcium salt of an acid (25%), and a syrup (60%) containing d-xylose and d-galactose.

4. The calcium salt appeared to be the calcium salt of an aldobionic acid and contained a methyl pentose residue which has not yet been identified.

5. More drastic hydrolysis of the polysaccharide led to the isolation of the barium salt of a uronic acid in poor yield. Since this acid on oxidation readily yielded mucic acid and had a specific rotation similar to that of d-galacturonic acid, which rapidly became negative on standing with 1% methyl-alcoholic hydrogen chloride, the uronic acid concerned appears to be d-galacturonic acid.

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EXPERIMENTAL

Part II

The Study of the fully methylated "free acid" Mucilage.

Attempted Direct Methylation of the "free acid" Mucilage.

The mucilage (5 g.) was suspended in water (100 c.c.) and stirred vigorously with a mechanical stirrer. Dimethyl sulphate (60 c.c.) and 30% sodium hydroxide (120 c.c.) were added simultaneously at 60° in one tenth portions every ten minutes. After all the reagents had been added the temperature was kept at 75°-80° for 1 hour. The whole was then cooled and neutralised carefully with glacial acetic acid. There was very little solid present, apart from a little sodium sulphate. The inorganic salts were now dialysed in parchment bags, a constant stream of water being caused to circulate round the bags. After 4-5 days there was no sulphate present so the resulting liquid was taken to dryness at 50°/15 mm. to give a celluloid-like product (3.4 g.), OMe 15.6%. The product was remethylated by the same method and worked up as before to give a solid (2.9 g.), OMe 20.7%. A third methylation yielded a product (2.7 g.), OMe 21.0%, and the methoxyl content was not increased by a fourth methylation.

Acetylation of the "free acid" Mucilage.

The appropriate method was found by three experiments,/

experiments, pyridine and acetic anhydride being the reagents employed.

I. The first method attempted was the one employed for the acetylation of agar (1). The dry mucilage (4 g.) was heated at 70° with pyridine (50 c.c.) for about 2 hours. The mucilage did not dissolve but dispersed into fine particles. The whole was cooled and the acetylating mixture (50 c.c. acetic anhydride + 20 c.c. pyridine) added slowly with shaking. No heat was evolved. The mixture was then heated on the boiling water bath for 24 hours, with periodic shaking. There still remained solid particles and the solution was very dark in colour. The whole was poured into a stream of cold water whence the acetate was precipitated as a brown cake. The excess reagents were removed by washing the product in muslin bags in a constant stream of water for 24 hours. The product was finally dried in the air (4.5 g.), CH_3CO 37%, and was only slightly soluble in acetone.

II. This method was very much the same as the above only the mucilage was not so thoroughly dried after its preparation, it being left slightly damp with alcohol and ether. Furthermore after the 24 hour's heating the resulting dark mixture was left for a further/

further 24 hours at room temperature with periodic shaking. The yield (4.7 g.) and product was much the same as in the last case, CH_3CO 38%.

The chief objection to these methods was the darkness of the solution after the long heating on the water bath. This always led to a brown-coloured acetate. The method described below in III was found to be much more suitable and was taken as the standard method.

III. The dry mucilage (30 g.) was shaken up vigorously with pyridine (300 c.c.) in the cold. When dispersion was complete, acetic anhydride (250 c.c.) was added in 50 c.c. portions with shaking. The mixture was then heated on the boiling bath for 2-3 hours only, the solution then becoming slightly brown in colour. It was then set aside for at least 48 hours with periodic shaking. The acetate was precipitated and purified as above and yielded a pale cream-coloured solid (35 g.). The product was fractionated by refluxing several times with a mixture of acetone and chloroform (1:1) to yield two fractions, a soluble one 'A' (14 g.) and a residue 'B' (20 g.). 'A' was isolated by pouring the concentrated extract into light petroleum (b.p. 60°-80°), the acetate being/

being precipitated as a cream powder.
Found for 'A'. CH_3CO. 41.0%. $[\alpha]_D^{18°}$ -72° (c, 0.3 in acetone).
Found for 'B'. CH_3CO. 36.0%.
The properties and acetyl content of 'B' were unchanged on reacetylation so that one acetylation appears to be sufficient. The fact that only 40% of the product is soluble in acetone may be due to the occurrence of the cellulosic part mentioned in the account of the acid hydrolysis.

Methylation of the soluble acetate 'A'.

The acetate (5 g.) was stirred vigorously with acetone (200 c.c.), a mechanical stirrer being employed. Dimethyl sulphate (100 c.c.) and 30% sodium hydroxide (250 c.c.) were added simultaneously in one tenth portions every 10 minutes at 40°-45°. After all the additions the temperature was raised slowly to remove the acetone and the solution was finally heated at 75°-80° for 1 hour. A brown solid separated along with some sodium sulphate. The whole was filtered hot. During the course of three or four of these trial experiments it was noticed that the solid remained hard and brittle if the filtration was rapid and the hot liquid was quickly removed. The sodium sulphate was not removed/

removed at this stage so the yield could not be observed; OMe ca. 25%. The whole of the solid material was put back again into the flask and remethylated as above. The resulting solution was again filtered hot and the bulk of the sodium sulphate removed by washing carefully with small amounts (100 c.c.) of boiling water, the product (2.5 g.) being immediately dried by suction; OMe ca. 30%. The product was again methylated and worked up to give the methylated mucilage (1.9 g.), OMe ca. 35%, which was methylated ~~again~~ for the fourth time. The residue after repeated washing with boiling water was dried and extracted with chloroform. The chloroform solution was dried over anhydrous sodium sulphate, concentrated to small bulk and poured into light petroleum (b.p. 60°-80°), giving a cream powder (1.5 g.), OMe 35%, $[\alpha]_D^{18°}$-100° (C, 0.7 in chloroform). It appeared therefore that three methylations were sufficient.

Methylation of the insoluble acetate 'B'.

The acetate (5. g) was methylated three times in exactly the same way as above and yielded a cream-coloured product (2.0 g.), OMe 34%, $[\alpha]_D^{18°}$-95° (C, 1.1 in chloroform). These constants were unchanged on further methylation.

Methylation/

Methylation of the crude, unfractionated acetate.

The acetate (5 g.) was methylated three times as above and gave a very similar product (2.0 g.), OMe 35%, $[\alpha]_D^{18°}$ -93° (C, 1.0 in chloroform).

Viscosity Determinations.

Although it is certain that the method of Staudinger is not strictly applicable to compounds of this type (in any event the true constant to use and the molecular weight of the repeating unit are unknown) it was thought that a comparison of the apparent molecular weights for such of the acetates and methylated products which were soluble in m-cresol would be of value. It was unfortunate that the insoluble acetate 'B' and the crude acetate were not sufficiently soluble in m-cresol for determinations.

Substance	Gt. in 100 c.c. m-cresol (c)	Time of flow (secs.)	Temp.	$\eta_{sp.}$	$\eta_{sp./c}$	$\eta_{sp./c'}$
m-cresol	-	449.01	18°	-	-	-
"	-	299.48	25°	-	-	-
Soluble acetate A.	0.311 g.	566.42	18°	0.262	0.842	18.2
"	"	370.20	25°	0.236	0.759	16.4
Methylated product from acetate A.	0.313 g.	612.07	18°	0.363	1.160	18.6
"	"	398.71	25°	0.331	1.058	16.9
Methylated product from acetate B.	0.307 g.	800.00	18°	0.782	2.550	40.8
"	"	513.37	25°	0.714	2.326	37.2
Methylated product from crude acetate	0.314 g.	804.97	18°	0.793	2.526	40.4
"	"	517.32	25°	0.728	2.320	37.1

C is the concentration of substance in g./100 c.c. m-cresol.

C' is the concentration in g.-mols. of acetylated or methylated anhydroxylose residue per litre, assuming the repeating unit to be a dihydroxy anhydroxylose, $C_5H_8O_4$ ·

Fractionation of the methylated product prepared from the crude acetate.

To the methylated polysaccharide (15 g.), OMe 34.7%, in chloroform (300 c.c.) light petroleum was added with vigorous stirring. After the addition of 750 c.c. the solution was centrifuged and the solid I (8.51 g.) dried in a vacuum. More light petroleum (300 c.c.) was added to the remaining solution and the solid portion II (3.95 g.) again removed and dried. The solution was concentrated to 100 c.c. and light petroleum (500 c.c.) again added to give more solid material III (2.45 g.). The remaining liquid on evaporation at 45°/20 mm. gave no residue.

Found:-

I. OMe 32.1% $[\alpha]_D^{18°}$ -95° (C, 1.0 in chloroform).

II OMe 33.3% $[\alpha]_D^{18°}$ -109° (C, 1.1 in chloroform).

III OMe 35.7% $[\alpha]_D^{18°}$ -110° (C, 1.2 in chloroform).

Attempted hydrolysis of the methylated mucilage.

As the methylated polysaccharide is insoluble in boiling water it is obvious that aqueous reagents will be unlikely to be very efficient. However a few were tried out to see the effect. 3%-Oxalic acid, 4%-sulphuric acid and 7%-hydrochloric acid were used. In each case the products dissolved very/

very slowly in the cold and an initial reading was just possible but when the solutions were put into the boiling water bath the methylated substance was deposited as a sticky gum and the hydrolysis proceeded only very slowly. 3% Methyl-alcoholic oxalic acid was then tried and looked more hopeful since the solid remained in solution on heating. The rotation however did not change even after three hours and the solution was non-reducing to Fehling's solution. It was decided, thereupon, to try methyl-alcoholic hydrogen chloride. A test hydrolysis on 0.25 g. showed that 3% methyl-alcoholic hydrogen chloride was an excellent reagent.

Typical hydrolysis of the methylated polysaccharide.

The methylated compound (8 g.) was refluxed on the water bath with 3% methyl-alcoholic hydrogen chloride (120 c.c.) until the rotation became constant (18 hours). $[\alpha]_D^{17^\circ}$ -95° (initial value); -33° (6 hours); $+39^\circ$ (12 hours) and $+97^\circ$ (18 hours). The cooled solution was neutralised with silver carbonate and the filtrate evaporated to give a non-reducing syrup (7.08 g.). This syrup was divided into four main fractions by vacuum distillation.

I/

I. 3.63 g., b.p. 80°-100°/0.02 mm. (bath temp.), $n_D^{19°}$ 1.4467, $[\alpha]_D^{18°}$ +69° (C, 1.0 in chloroform), OMe 50.2%.

II. 0.50 g., b.p. 100°-115°/0.04 mm. (bath temp.), $n_D^{19°}$ 1.4571, $[\alpha]_D^{18°}$ +70° (C, 1.0 in chloroform), OMe 46.5%.

III. 0.74 g., b.p. 115°-135°/0.04 mm. (bath temp.), $n_D^{19°}$ 1.4655, $[\alpha]_D^{18°}$ +76° (C, 0.9 in chloroform), OMe 43.1%.

IV. 1.96 g., b.p. 140°-180°/0.04 mm. (bath temp.), $n_D^{19°}$ 1.4737, $[\alpha]_D^{18°}$ +84° (C, 1.2 in chloroform), OMe 35.4%.

The undistilled residue weighed 0.25 g.

These were typical fractions obtained in the early part of the work. Fraction I was thought, from the data, to be a dimethyl methyl-xyloside, so it was decided to methylate a part of this fraction completely.

Methylation of Fraction I.

Fraction I (0.75 g.) was fully methylated by Purdie's method and the product (0.80 g.) distilled to give a colourless, mobile oil (0.63 g.), b.p. 70°-90°/0.03 mm. (bath temp.), $n_D^{20°}$ 1.4397, $[\alpha]_D^{19°}$ +50° (C, 0.5 in chloroform), OMe 56.5% (calc. for $C_9H_{18}O_5$ OMe 60.1%).

The/

Hydrolysis of the fully methylated oil.

The oil (0.70 g.) was hydrolysed with 2% nitric acid (25 c.c.) on the boiling water bath until the rotation became constant (1½ hours). $[\alpha]_D^{18^\circ}$ + 50° (initial value) and + 27° (after 1½ hours: constant value). The cooled solution was neutralised with barium carbonate and heated to remove any bicarbonate. The filtrate was concentrated at 50°/15 mm. and the resulting solid extracted exhaustively with boiling ether to give a viscous syrup (0.56 g.) which crystallised overnight, m.p. 90°, after recrystallisation from ether. Mixed m.p. with an authentic sample of 2:3:4-trimethyl xylose 91°-92°. $[\alpha]_D^{20^\circ}$ + 55.5° → + 27.6° (c, 0.5 in water), OMe 46.3% (calc. for $C_8H_{16}O_5$ OMe 48.4%).

This proved that fraction I was a xylose derivative but it was still thought to be entirely a dimethyl xylose. It was therefore decided to try to prepare an anilide from it since 2:3-dimethyl xylose anilide is known. Fraction I (2.0 g.) was therefore hydrolysed to the free sugar with 2% nitric acid (50 c.c.). $[\alpha]_D^{18^\circ}$ + 69° (initial value) and + 24° (2 hours; constant). The solution was worked up as/

as above to give a viscous syrup (1.7 g.) which on standing commenced to crystallise. Some of the crystals were tiled and gave a m.p. of 85°. A mixed m.p. with crystals of 2:3:4-trimethyl xylose showed no depression, so it was concluded that the crystals were probably 2:3:4-trimethyl xylose. Fraction I was therefore in all probability a mixture and required further fractionation.

At this point it will be convenient to give the data for the hydrolyses of fractions I and II of the methylated polysaccharide (p.61) as they form a more typical example of the hydrolysis of the fully methylated mucilage.

Hydrolysis of fraction I of the methylated polysaccharide (p.61).

Fraction I (9.37 g.) was boiled with 3% methyl-alcoholic hydrogen chloride (200 c.c.) until the rotation became constant (17 hours). $[\alpha]_D^{18°}$ -95° (initial value) and +90° (17 hours; constant value). The cooled solution was neutralised with silver carbonate and the filtrate concentrated to yield a non-reducing syrup (9.25 g.) which on vacuum distillation gave:-

I 3.26 g., b.p. 80°-100°/0.03 m.m. (bath temp.), $n_D^{15°}$ 1.4477, $[\alpha]_D^{16°}$ +49° (c, 0.8 in water), OMe 54.3%.

II 1.20 g., b.p. 100°-115°/0.04 m.m. (bath temp.), $n_D^{15°}$ 1.4533, $[\alpha]_D^{16°}$ +67° (c, 1.0 in water), OMe 52.2%.

III/

III 2.10 g., b.p. 115°-140°/0.04 m.m. (bath temp.), $n_D^{15°}$ 1.4636, $[\alpha]_D^{16°}$ +77° (C, 1.0 in water), OMe 44.4%.

IV 2.11 g., b.p. 140°-190°/0.05 m.m. (bath temp.), $n_D^{15°}$ 1.4758, $[\alpha]_D^{16°}$ +90° (C, 1.1 in chloroform), OMe 39.1%.

The undistilled residue weighed 0.58 g.

Fractions I and II were re-fractionated, using a Claisen flask fitted with a vacuum-jacketed condenser, to give:-

Ia. 2.07 g., b.p. 63°-65°/0.03 m.m. $n_D^{12°}$ 1.4433.

Residue IIa. 2.39 g., $n_D^{12°}$ 1.4550.

Hydrolysis of fraction II of the methylated polysaccharide (P.61).

The product (8.14 g.) was treated with 3% methyl-alcoholic hydrogen chloride (200 c.c.) as above to yield a non-reducing syrup (8.32 g.) which after distillation and subsequent re-fractionation as before gave:-

Ia 2.52 g., b.p. 90°-100°/0.02 m.m. (bath temp.), $n_D^{14°}$ 1.4417.

IIa 2.31 g., b.p. 100°-120°/0.02 m.m. (bath temp.), $n_D^{14°}$ 1.4555.

III 1.86 g., b.p. 120°-140°/0.03 m.m. (bath temp.), $n_D^{14°}$ 1.4675.

IV 1.40 g., b.p. 140°-190°/0.04 m.m. (bath temp.), $n_D^{14°}$ 1.4727.

The undistilled residue weighed 0.23 g.

The rest of the work of this thesis deals with the attempts to characterise these fractions. The above scheme was adhered to as closely as possible when preparing more material.

The Study of Fraction Ia.

The analyses figures obtained were:-

$n_D^{17°}$ 1.4406 (authentic 2:3:4-trimethyl methylxyloside gave $n_D^{17°}$ 1.4403), $[\alpha]_D^{20°}$ +46° (C, 0.5 in chloroform), OMe 58.7% (calc. for $C_9H_{18}O_5$ OMe 60.1%).

Hydrolysis of fraction Ia.

The mobile oil (0.5 g.) was heated on the boiling water bath for 1 hour with 2% nitric acid (15 c.c.). The solution was neutralised with barium carbonate and worked up to give a solid which on repeated extractions with boiling ether gave a viscous syrup (0.45 g.) which on standing in the refrigerator crystallised completely in a few days (0.43 g.). The crystalline material had:-

M.p. 89°; mixed m.p. with an authentic specimen of 2:3:4-trimethyl xylose 90°. $[\alpha]_D^{20°}$ +55° → +30° (C, 0.7 in water), OMe 47.4% (calc. for $C_8H_{16}O_5$ OMe 48.4%).

It is clear, therefore, that fraction Ia is composed entirely of 2:3:4-trimethyl methylxyloside.

The Study of Fraction IIa/

The Study of Fraction IIa.

This fraction had:-
$n_D^{15°}$ 1.4555, $[\alpha]_D^{18°}$ +69° (C, 1.0 in chloroform), OMe 47.7%. These figures indicate a dimethyl methylxyloside, and the only known dimethyl methylxyloside is the 2:3-derivative isolated from dimethyl xylan (2). This compound was reported to give a crystalline anilide so it was decided to try an anilide preparation on the sugar obtained on the hydrolysis of fraction IIa. It was necessary however to prove that the fraction was really a xylose derivative by the isolation of the crystalline 2:3:4-trimethyl xylose on methylation and subsequent hydrolysis.

Methylation of fraction IIa.

The mobile syrup (1.0 g.) was methylated twice by Purdie's method to yield a mobile oil (1.09 g.), $n_D^{17°}$ 1.4410.

Hydrolysis.

The above product (1.07 g.) was hydrolysed in the usual way with 2% nitric acid (25 c.c.). The solution was worked up as before and the resulting solid exhaustively extracted with boiling ether to yield a viscous syrup (0.87 g.) which crystallised completely on standing. M.p. 89°, mixed m.p. with an authentic specimen of 2:3:4-trimethyl xylose 90°.

$[\alpha]_D^{18°}$ +53° → +27° (C, 1.0 in water), OMe 46.7% (calc. for $C_8H_{16}O_5$ OMe 48.4%).

The crystalline material was, therefore, 2:3:4-trimethyl xylose and fraction IIa is definitely proved to be a xylose derivative.

Attempted anilide formation from fraction IIa.

The dimethyl methyl xyloside (0.5 g.) was subjected to the usual treatment with 2% nitric acid (15 c.c.) for 1 hour, in order to prepare the free sugar. On working up the solution a viscous syrup (0.42 g.) was obtained, $n_D^{14°}$ 1.4750 $[\alpha]_D^{16°}$ +27° (C, 1.0 in water), OMe 32.4% (Calc. for $C_7H_{14}O_5$ OMe 34.8%). This syrup has failed to yield crystals after standing for a year. To the syrup (0.3 g.) in alcohol (5 c.c.) freshly distilled aniline (0.2 g.) was added. The mixture was heated on the water bath for 1 hour and then allowed to cool. No crystals appeared. The solvent was therefore removed in a vacuum but the resulting syrup failed to give crystals even on standing for a year. This experiment was repeated seven times with slight variation of the conditions without result. For purposes of comparison it was decided at this point to prepare some 2:3-dimethyl methylxyloside from dimethyl xylan.

Preparation of 2:3-dimethyl methylxyloside.

Dimethyl/

Dimethyl xylan (7 g.) prepared from xylan by the method of Haworth, Hirst and Percival (3), was boiled for 11 hours with 1.2% methyl-alcoholic hydrogen chloride (200 c.c.), the specific rotation then reaching a constant value of $[\alpha]_D^{16°}$ +54° as quoted by Hampton in his paper (2). The solution was neutralised with silver carbonate and worked up to give a non-reducing syrup (6.83 g.) which on vacuum distillation gave:-

I 0.26 g., b.p. 90°-95°/0.03 m.m. (bath temp.), $n_D^{18°}$ 1.4444, $[\alpha]_D^{17°}$ -25° (C, 0.5 in chloroform).

II 2.53 g., b.p. 100°-110°/0.02 m.m. (bath temp.), $n_D^{18°}$ 1.4540, $[\alpha]_D^{18°}$ +45° (C, 1.0 in chloroform).

III 3.56 g., b.p. 110°-130°/0.03 m.m. (bath temp.), $n_D^{15°}$ 1.4578 $[\alpha]_D^{18°}$ +70° (C, 1.0 in chloroform).

The undistillable residue weighed 0.48 g.

Fractions II and III were the required dimethyl methylxyloside.

Preparation of 2:3-dimethyl xylose anilide.

The dimethyl $\overset{\text{methyl}}{\wedge}$xyloside (1.0 g.) was converted into the free sugar by treatment with 2% nitric acid and gave a viscous syrup (0.8 g.) which was treated with aniline as described above. On cooling no crystals were deposited. The alcohol was removed as before and the resulting syrup solidified completely after a fortnight. M.p. 143° on recrystallisation from ethyl acetate. cf. (2).

Preparation of 2:3-dimethyl-γ-xylonolactone.

2:3-Dimethyl methylxyloside (0.75 g.) was hydrolysed with 2% nitric acid (15 c.c.) in the usual way to yield a viscous syrup (0.68 g.). This product was taken up in water (10 c.c.) and liquid bromine (1 c.c.) added, the whole being kept at 40° until the reducing action had disappeared (2 days). The excess of bromine was removed by aeration, the solution neutralised with silver carbonate and the silver ions removed with hydrogen sulphide. The excess of hydrogen sulphide was removed by aeration and the solution finally concentrated to a glass (0.5 g.). This product was heated at 100°/15 m.m. for 3 hours and then exhaustively extracted with ether to yield a colourless syrup (0.28 g.). It was thought that the residue contained the sodium salt of the sugar acid due to impurities in the silver carbonate. The calculated amount of dilute hydrochloric acid was therefore added to the residue (assumed to be the sodium salt). The solution was concentrated at 40°/15 m.m. to 5 c.c. and distilled water added. The solution was evaporated in this way twelve times, and finally concentrated to a syrup which was again heated at 100°/15 m.m. for 3 hours. Extraction with boiling ether yielded

a/

a further quantity of impure lactone (0.12 g.). This was combined with the original yield and distilled to give a colourless syrup (0.36 g.), b.p. 130°-140°/ 0.03 m.m. (bath temp.), $n_D^{14°}$ 1.4621.

Hydrolysis of 2:3-dimethyl- γ -xylonolactone.

$[\alpha]_D^{16°}$ in water (c, 0.453) +95° (5 mins.), +88.3° (24 hours), +81.7° (96 hours), +77.3° (144 hours), +72.8° (288 hours), +70.6° (384 hours), +68.5° (500 hours; constant value).

Titration of the lactone with N/20 sodium hydroxide.

2.86 C.c. of the above solution (immediately on solution) were titrated with N/20 sodium hydroxide. The neutralisation was slow even on warming the solution. Volume of N/20 sodium hydroxide used was 1.50 c.c. (calc. for $C_7H_{12}O_5$ 1.47 c.c. required).

Preparation of the lactone from the unknown dimethyl methylxyloside.

The dimethyl xyloside (0.7 g.) was treated as above with 2% nitric acid to yield a viscous syrup (0.6 g.), which was treated with bromine as in the case of the 2:3-derivative. The solution on working up yielded again a glass (0.35 g.) which was heated at 100°/15 m.m. for 3 hours. Repeated extraction with boiling ether yielded only a small amount/

amount of syrup so the hydrochloric acid treatment was again performed. This gave a further yield of syrup although the yield was much poorer than in the case of the 2:3-derivative. The yields were combined and distilled in vacuum to give a colourless syrup (0.15 g.), b.p. 140°/0.03 m.m. (bath temp.), $n_D^{14°}$ 1.4600, OMe 35.2% (calc. for $C_7H_{12}O_5$ OMe 36.1%). On standing this lactone began to crystallise. M.p. 67°.

Hydrolysis of the lactone.

$[\alpha]_D^{18°}$ in water (C, 0.413) +41.2° (5 minutes), +36.3° (2 hours), +31.5° (4 hours), +31.3° (6 hours; constant value).

Titration of the lactone with N/20 sodium hydroxide.

2.50 C.c. of the above solution were titrated with N/20 sodium hydroxide as before. The neutralisation was very rapid even in the cold. Volume of N/20 sodium hydroxide required 1.12 c.c. (calc. for $C_7H_{12}O_5$ 1.17 c.c.).

The preparation of the above lactone was repeated with similar results. The syrup (0.0828 g.) required 8.66 c.c. of N/20 sodium hydroxide for neutralisation. (Calc. for $C_7H_{12}O_5$ 9.41 c.c.).

$[\alpha]_D^{20°}$ in water (C, 1.063) +40.5° (initial value) and +30.9° (6 hours; constant value).

Preparation of 2:3-dimethyl xylonamide.

The syrupy lactone (0.1 g.) was treated with dry concentrated methyl-alcoholic ammonia (2 c.c.) at 0° for 2 days. The solvent was then removed in a vacuum to yield a white solid, m.p. 131°, $[\alpha]_D^{14°}$ +47.6° (C, 0.4 in water). cf. Bywater, Haworth, Hirst and Peat (4).

Preparation of the amide of the unknown dimethyl xylonolactone.

The unknown lactone (0.10 g.) was treated exactly as above. On removing the solvent a syrupy amide was obtained which did not crystallise even on standing for several months. $[\alpha]_D^{14°}$ +54° (C, 0.8 in water).

WEERMAN TESTS.

(1) Gluconamide. The amide (0.0518 g.) was dissolved in water (1 c.c.), sodium hypochlorite solution (0.7 c.c.) added and the mixture kept at 0° for 3 hours. The excess of hypochlorite was destroyed with sodium thiosulphate and the solution saturated with sodium acetate. A saturated solution of semicarbazide hydrochloride was added and a white precipitate of hydrazodicarbonamide (0.0135 g.) rapidly settled out. m.p. 254°-256° d.

(2)/

(2) 2:3-dimethyl xylonamide. The amide (0.03 g.) was dissolved in water (1 c.c.) and the same procedure repeated. No precipitate was obtained even on standing overnight.

(3) Unknown dimethyl xylonamide. The amide (0.0305 g.) was treated in exactly the same way as above. A white precipitate of hydrazodicarbonamide (0.0111 g.) was obtained, m.p. 257° d. and mixed m.p. with an authentic specimen of hydrazodicarbonamide 252°-254° d.

It is obvious from these results that position 2 is free and that the xylose derivative is probably 3:4-dimethyl xylose. Confirmation of this idea was sought as follows:-

Attempted preparation of an osazone from the unknown dimethyl xylose.

The free dimethyl sugar (0.3 g.), prepared in the usual way from the xyloside was dissolved in water (10 c.c.) containing glacial acetic acid (2 c.c.). Phenylhydrazine (0.5 g.) and a little sodium bisulphite were added and the whole heated on the water bath at 90° for 1 hour and then allowed to cool slowly. A tar separated out. The solution was poured off and reheated as above. On cooling more tar was obtained. Five such yields were obtained. The tars/

tars were then dissolved in ether, the solution dried thoroughly with sodium sulphate, concentrated to small bulk and poured into an excess of light petroleum (b.p. 60°-80°), whence a brown solid was obtained. This was removed on the centrifuge and dried in a vacuum (0.10 g.). It was found impossible to purify it further. OMe 13.2% (calc. for $C_{19}H_{24}O_3N_4$ OMe 17.4%).

Oxidation of the dimethyl methylxyloside with nitric acid.

Two oxidations were performed, the second being slightly more drastic than the first.

I. The xyloside (0.9 g.) was dissolved in concentrated nitric acid (10 c.c.) and heated at 50° until the evolution of brown fumes had ceased (1-2 hours). The temperature was then raised slowly and kept at 90° for a further 4 hours. The solution was diluted to twice its volume with water and concentrated at 40°/15 m.m. to 5 c.c. More water was added and the whole again evaporated to 5 c.c. This treatment was repeated twelve times. The solution was finally concentrated to a syrup which was esterified by boiling with 3% methyl-alcoholic hydrogen chloride (50 c.c.) for 6 hours. The acid was neutralised with/

with silver carbonate and the solution worked up to give a viscous syrup (0.8 g.) which on distillation gave:-

I 0.30 g., b.p. 120°-125°/0.04 m.m. (bath temp.), $n_D^{13°}$ 1.4459, $[\alpha]_D^{15°}$ +45° (c, 0.6 in methyl alcohol), OMe 55.2%, COOMe 53.6% (calc. for hydroxydimethoxy methyl glutarate $C_9H_{16}O_7$ OMe 52.5%, COOMe 50.0%).

II 0.45 g., b.p. 130°-150°/0.05 m.m. (bath temp.), $n_D^{13°}$ 1.4458, $[\alpha]_D^{15°}$ +41° (c, 0.8 in methyl alcohol), OMe 53.7%.

It was concluded that I and II were essentially a hydroxydimethoxy xyloglutaric ester.

Preparation of the amide of the hydroxy ester.

The syrupy ester (0.1 g.) was treated with dry concentrated methyl-alcoholic ammonia (1 c.c.) at 0° for 3 days. On removing the solvent a syrup was obtained in which there were a few crystals, m.p. 270° d., possibly d- or l-dimethoxy succinamide. The main product was an uncrystallisable syrup, $[\alpha]_D^{12°}$ +26.8° (c, 0.9 in water).

The amide (0.0571 g.) was treated in the usual way with sodium hypochlorite etc. (p.74) and gave a white precipitate of hydrazodicarbonamide (0.0102 g.), m.p. 254° d./

m.p. 254° d. showing the presence of a free hydroxyl group in an α-position.

Methylation of the hydroxy ester.

The hydroxy ester (0.4 g.) was twice methylated by Purdie's method to yield a product which was distilled to give a clear, mobile syrup (0.32 g.), b.p. 110°-115°/0.02 m.m. (bath temp.), $n_D^{11°}$ 1.4437, $[\alpha]_D^{11°} \pm 0$ (C, 1.5 in methyl alcohol), OMe 59.7% (calc. for $C_{10}H_{18}O_7$, OMe 62.0%).

Isolation of i-xylo-trimethoxy glutaramide.

The fully methylated ester (0.1 g.) was treated with dry methyl-alcoholic ammonia (2 c.c.) for 3 days. The solvent was removed and the resulting syrup allowed to stand overnight after which it had crystallised completely. A bluish-green colour developed and remained on the crystals for some days, eventually fading in about a fortnight's time c.f. Hirst and Purves (5). M.p. of the crystalline material, sintered 183° and melted 194° and mixed m.p. with an authentic specimen of i-xylo-trimethoxy glutaramide 193°. $[\alpha]_D^{16°} \pm 0$ (C, 1.2 in water). Found C, 44.10%; H, 7.00%; OMe 40.9% (calc. for $C_8H_{16}O_5N_2$; C, 43.6%; H, 7.27%; OMe 42.3%).

II. In the second experiment the oxidation was carried out for a longer period. The dimethyl methylxyloside/

methylxyloside (0.7 g.) was heated as before at 50° with concentrated nitric acid (10 c.c.) for 2 hours and finally heated to 90° for a further 7 hours. The product was worked up and put through exactly the same processes as before. The resulting syrup was distilled to give a pale yellow viscous syrup (0.25 g.) b.p. 120°-140°/0.05 m.m. (bath temp.), $n_D^{10°}$ 1.4495, $[\alpha]_D^{12°}$ +40° (C, 1.0 in methyl alcohol).

Amide Formation.

As before the hydroxy ester (0.1 g.) was converted into the amide with methyl-alcoholic ammonia (1 c.c.). When the solvent was removed it was noticed that although the bulk of the amide was syrupy numerous crystals had separated, m.p. 270°-280° d., and were apparently either d- or l-dimethoxy succinamide. Mixed m.p. with an authentic sample of d- dimethoxy succinamide 254°-257° d. $[\alpha]_D^{17°}$ -90° (C, 0.3 in water). The main product was again however syrupy, and gave a +ve Weerman.

Oxidation of authentic 2:3:4-trimethyl xylose with nitric acid.

For purposes of comparison 2:3:4-trimethyl xylose (0.5 g.) was oxidised as in experiment I above, to give on distillation a clear, viscous syrup (0.33 g.), b.p. 110°-120°/0.02 m.m. (bath temp.), $n_D^{13°}$ 1.4437, $[\alpha]_D^{14°} \pm 0$ (C, 2.0 in methyl alcohol), OMe 60.2% (Calc. for $C_{10}H_{18}O_7$; OMe 62.0%).

Preparation/

Preparation of i-xylo-trimethoxy glutaramide.

Half of the above inactive ester (0.2 g.) was converted into the amide which crystallised overnight. M.p. 192°, mixed m.p. with an authentic specimen of i-xylo-trimethoxy glutaramide 193°. $[\alpha]_D^{15°} \pm 0$ (c, 1.2 in water), OMe 40.3% (calc. for $C_8H_{16}O_5N_2$ OMe 42.3%).

The Study of Fraction III.

This fraction gave:-
$n_D^{16°}$ 1.4635, $[\alpha]_D^{17°} +75°$ (c, 0.7 in chloroform), OMe 43.3%.
A preliminary test indicated the presence of a small amount of a carbomethoxy residue.

Hydrolysis of Fraction III.

The syrup (0.54 g.) was heated on the boiling water bath with 7% hydrochloric acid (50 c.c.) until the rotation became constant (6 hours). $[\alpha]_D^{15°} + 73.6°$ (initial value), $+ 65.2°$ (1 hour), $+ 52.4°$ (3 hours), and $+35.2°$ (6 hours; constant value). The cooled solution was neutralised with silver carbonate, the silver ions removed with hydrogen sulphide and the solution concentrated to 30 c.c. Neutralisation was then effected with barium carbonate, the solution being heated on the water bath to decompose any bicarbonate. After filtration and evaporation a viscous syrup was obtained which was exhaustively extracted/

extracted with boiling ether. The solution on evaporation yielded a syrup (0.39 g.), the residue being a glass (0.05 g.). In later experiments products were obtained which were entirely soluble in ether, showing that the uronic acid cannot be present in quantity in fraction III.

Found for the syrup. $[\alpha]_D^{14^\circ}$ +22.8° (C, 0.6 in water), OMe 36.2, calc. for $C_7H_{14}O_5$ OMe 34.8%.

Found for the glass. OMe 17.2%.

Even on standing for several months the syrup did not crystallise.

Attempted lactone preparation.

The above syrup (0.25 g.) dissolved in water (10 c.c.) was oxidised with bromine (1 c.c.) in the usual way. On distillation a viscous syrup (0.15 g.) was obtained, b.p. 150°/0.04 m.m. (bath temp.), $n_D^{14^\circ}$ 1.4693. (Found, OMe 37.2, calc. for $C_7H_{12}O_5$: OMe 36.1%).

Hydrolysis of the lactone.

$[\alpha]_D^{16^\circ}$ in water (C, 3.790) + 50.1° (5 minutes), +48.6° (1 hour), + 45.3° (3 hours), + 40.0° (7 hours; constant value).

Titration of the lactone.

The syrup (0.1132 g.) required 12.01 c.c. of N/25 sodium hydroxide (calc. for $C_7H_{12}O_5$. 11.90 c.c.), the/

the neutralisation being quite rapid even in the cold.

Amide Formation.

The lactone (0.1 g.) was converted into the amide by treatment with methyl-alcoholic ammonia (1 c.c.). The product was a syrup and gave a positive Weerman test.

Methylation of Fraction III.

The syrup (0.2 g.) was methylated three times by Purdie's method and the resulting syrup distilled to give a clear, mobile oil (0.21 g.), b.p. 90°-100°/ 0.02 m.m. (bath temp.), $n_D^{15°}$ 1.4430, $[\alpha]_D^{14°}$ +4.2° (c, 0.9 in chloroform), (Found, OMe 61.1, calc. for $C_9H_{13}O_5$: OMe 60.1%).

Hydrolysis.

The oil (0.17 g.) was hydrolysed with 7% hydrochloric acid (10 c.c.) at 95° until the rotation became constant (1½ hours). $[\alpha]_D^{15°}$ +4.2° (initial value) and +41.1° (1½ hours; constant value). The solution was neutralised with barium carbonate, evaporated to dryness and the resulting solid exhaustively extracted with boiling chloroform to yield a viscous syrup (0.12 g.) which was treated with aniline (0.05 g.) in alcohol (5 c.c.) in the usual way. A crystalline anilide (25 mg.) was obtained, m.p. 197° on recrystallisation/

recrystallisation from ethyl acetate. Mixed m.p. with an authentic specimen of 2:3:4:6-tetramethyl galactose anilide 196°. $[\alpha]_D^{14°}$ +62° (C, 0.4 in ethyl acetate).

Found;- C, 61.6 ; H, 8.2 ; N, 4.55 ; OMe 38.6

Calc. for $C_{16}H_{25}O_5N$: C, 61.7 ; H, 8.0 ; N, 4.5 OMe 39.8%.

Methylation of Fraction III repeated.

Fraction III (0.52 g.) was completely methylated as above to give a mobile syrup (0.63 g.), b.p. 100°/ 0.02 m.m. (bath temp.), $n_D^{14°}$ 1.4450, $[\alpha]_D^{15°}$ +63.6° (C, 0.9 in water).

Found, OMe 62.0. calc. for $C_9H_{18}O_5$: OMe 60.1%

Hydrolysis.

The mobile syrup (0.59 g.) was hydrolysed with 7% hydrochloric acid (20 c.c.) until the rotation became constant (1½ hours). $[\alpha]_D^{14°}$ +63.6° (initial value) and +36.7° (1½ hours; constant value). The solution was worked up as before to yield a clear, viscous syrup (0.45 g.). After standing for a few days this product began to crystallise, m.p. 89°, mixed m.p. with an authentic specimen of 2:3:4-trimethyl-xylose 91°. Found, OMe 48.1. calc. for $C_8H_{16}O_5$: OMe 48.4% . $[\alpha]_D^{15°}$ + 56.2° ⟶ +33.3° (C, 0.7 in water). The yield of crystalline material was 0.34 g.

Anilide Formation /

Anilide Formation.

A portion of the above syrup (0.1 g.) was treated with aniline in the usual way to give a crystalline anilide (0.03 g.), m.p. 196°, mixed m.p. with an authentic specimen of 2:3:4:6-tetramethyl galactose anilide 197°.

Found, OMe, 38.9. calc. for $C_{16}H_{25}O_5N$; OMe 39.8%

The Study of Fraction IV.

This fraction had:-

$n_D^{15°}$ 1.4737, $[\alpha]_D^{18°}$ +90° (C, 0.8 in chloroform), OMe 39.3%.

A portion of this fraction (0.02 g.) was heated at 100° for 1 hour with barium hydroxide (0.03 g. in 5 c.c. of water), the excess of barium hydroxide removed with carbon dioxide and bicarbonate decomposed by vigorous boiling. A little dilute sulphuric acid was added to the filtered solution giving a white precipitate which indicated the presence of a carbomethoxy residue.

Hydrolysis of Fraction IV.

The viscous syrup (0.22 g.) was hydrolysed with 7% hydrochloric acid (30 c.c.) at 95° until the rotation became constant (2 hours). $[\alpha]_D^{17°}$ +87.8° (initial value), + 53.2° (½ hour), +44.6° (1 hour), +38.9° (2 hours ; constant value). The cooled solution/

solution was treated in exactly the same way as fraction III (p.82) to give a glass (0.18 g.) which on exhaustive extraction with boiling ether yielded two fractions namely a soluble viscous syrup and a residual glass. The experiment was then repeated on a larger scale to yield a soluble viscous syrup 'A' (1.8 g.) and an insoluble barium salt 'B' (0.53 g.).

Found for 'A'. $[\alpha]_D^{15^\circ}$ +44.3° (C, 0.7 in water), OMe 27.7%.

Found for 'B'. $[\alpha]_D^{15^\circ}$ +42.9° (C, 0.4 in water), OMe 17.7%. calc. for $C_8H_{13}O_7 \supset \frac{1}{2}Ba$: 21.3% .

Study of the soluble syrup 'A'.

A portion of 'A' (0.2 g.) was methylated four times with methyl iodide and silver oxide to give a mobile syrup (0.23 g.), which was hydrolysed in the usual manner with 7% hydrochloric acid. The resulting viscous syrup (0.17 g.) was treated with aniline (0.07 g.) and alcohol (5 c.c.) in the usual way to yield a crystalline anilide (0.03 g.), m.p. 195°, mixed m.p. with an authentic specimen of 2:3:4:6-tetramethyl galactose anilide 197°.

Anilide Formation.

Another portion of 'A' (0.15 g.) was treated with aniline/

aniline in the usual way. The resulting syrup crystallised after a few days to give long needles (15 mg.), m.p. 169° on recrystallisation from acetone. The quantity was insufficient for more than one recrystallisation. Mixed m.p. with an authentic specimen of 2:3:4-trimethyl galactose anilide (m.p. 167°) 149°; mixed m.p. with an authentic specimen of 2:4:6-trimethyl galactose anilide (m.p. 172°) 170°.

Found. OMe 29.5. calc. for $C_{15}H_{23}O_5N$: OMe 31.3%

Study of the barium salt 'B'.

Attempted glycoside formation in the cold (p.46).

$[\alpha]_D^{16°}$ in 1% methyl-alcoholic hydrogen chloride (c, 0.42) +42.1° (5 minutes), +40.0° (2 hours), +37.3° (7 hours), +28.2° (21 hours) +22.0° (4 days; constant value). The resulting solution was non-reducing to Fehling's solution.

Methylation of 'B'.

The remainder of this product (0.5 g.) was boiled with 3% methyl-alcoholic hydrogen chloride (50 c.c.) for 6 hours. The solution was neutralised with silver carbonate and worked up to give a viscous syrup (0.52 g.) which was methylated twice with methyl iodide and silver oxide. The product on distillation gave a colourless, mobile syrup 'C' (0.6 g.), b.p. 90°-110°/0.03 m.m. (bath temp.), $n_D^{14°}$ 1.4430 /

$n_D^{14^\circ}$ 1.4430, $[\alpha]_D^{15^\circ}$ +59.1° (c, 0.6 in water).

Found. OMe 57.2. calc. for $C_{11}H_{20}O_7$ OMe 58.7%.

This mobile syrup (0.1 g.) failed to yield a crystalline amide.

Oxidation of 'C.'

The product (0.5 g.) was converted into the free sugar by treatment with 7% hydrochloric acid (25 c.c.) for 1½ hours. The solution was neutralised with barium carbonate and the filtrate evaporated at 50°/15 m.m. to yield a viscous syrup (0.4 g.) which was oxidised with bromine in the usual way. The solution was worked up as before and the resulting mobile syrup esterified by boiling with 3% methyl-alcoholic hydrogen chloride (50 c.c.) for 6 hours. The product on distillation gave a colourless, mobile syrup (0.35 g.), b.p. 140°/0.05 m.m. (bath temp.), $n_D^{16^\circ}$ 1.4557. Found OMe 53.3 calc. for $C_{11}H_{20}O_8$: OMe 55.4%. This product failed to crystallise.

Discussion of Results

In order to ascertain the structure of the mucilaginous polysaccharide obtained from rib grass seed, the classical method of complete methylation followed by hydrolysis and the attempted separation of the fragments was employed. Direct methylation having proved unsuccessful the acetate was prepared by the treatment of the "free acid" mucilage with pyridine and acetic anhydride. The product was not completely soluble in acetone and chloroform, 60% of it remaining as an insoluble residue. The soluble portion was obtained either as a tough colourless glass from chloroform solution or as a cream powder by precipitation from this solution by light petroleum. The insoluble portion was obtained as a brownish powder the acetyl content of which was 5% lower than that of the soluble fraction. Further acetylation had no effect on the properties of either of the fractions. Each of the fractions was methylated three times with 30% sodium hydroxide solution and dimethyl sulphate and each yielded a product which was soluble in chloroform and acetone and which remained unchanged on further methylation. The crude, unfractionated acetate yielded on methylation/

methylation an apparently similar product which on fractional precipitation from chloroform with light petroleum gave three almost identical fraction indicating the essentially homogeneous character of the methylated polysaccharide. Viscosity determinations in m-cresol seemed to show however that the methylated polysaccharide derived from the crude acetate and the insoluble acetate was more complex than that obtained from the soluble acetate. These facts together with the difference in solubility of the acetates may have been connected with the presence of the supposed cellulosic portion mentioned in the account of the acid hydrolysis (p.49). The occurrence of cellulose in conjunction with polysaccharides of this type is quite common (p.17) (6).

The methylated polysaccharide was hydrolysed completely in 18 hours on boiling with 3% methyl-alcoholic hydrogen chloride. The non-reducing syrup produced was divided into four fractions on distillation in a high vacuum. Fractions I and II were mixtures and were refractionated to yield fractions Ia and IIa.

Fraction Ia (22%), $n_D^{17^\circ}$ 1.4406, OMe 58.7%. Fraction IIa/

IIa (25%), $n_D^{15°}$ 1.4555, OMe 47.7% Fraction III (22%), $n_D^{16°}$ 1.4655, OMe 43.3%. Fraction IV (23%), $n_D^{17°}$ 1.4737, OMe 39.3%.

The percentage yield was calculated on the weight of the methylated polysaccharide used.

Fraction Ia from its physical properties and analytical composition appeared to be a mixture of 2:3:4-trimethyl methylxylosides. Hydrolysis with 2% nitric acid led to the isolation of crystalline 2:3:4-trimethyl-α-d-xylose in good yield.

The presence of such a high proportion (a mean value of 22% from four fractions of the methylated mucilage) of trimethyl xylopyranose in the fragments obtained on hydrolysis is worthy of note. Since xylose is obtained so readily on hydrolysis of the mucilage with dilute acids (and even on heating with water) one might conclude that the trimethyl methyl-xylopyranoside was produced by fission during acetylation and methylation. This seems highly improbable since in the first instance the acetylated polysaccharide is non-reducing, and the fractionation of the methylated polysaccharide gave no indication of such an extensive degradation.

It seems necessary to conclude therefore that these xylose residues must exist in the mucilage as "end groups" (7) (8). Since little is known about the/

the constitution of the rest of the molecule it is clearly not of great value to speculate as to how these terminal pentose units are linked, especially since no monomethyl pentoses nor dimethyl hexoses have been identified. It appears permissable to suggest however that these xylopyranose residues are the terminal groups of side chains especially vulnerable to hydrolytic action. Fraction IIa was obviously a partially methylated derivative. Several methylations with silver oxide and methyl iodide yielded a product very similar to Ia. Subsequent hydrolysis with 2% nitric acid gave a a good yield of crystalline 2:3:4-trimethyl xylose. IIa was therefore a xylose derivative. The only dimethyl xylose which has been studied is 2:3-dimethyl xylose (2) which gives a crystalline anilide. Eight attempts to obtain a crystalline anilide from the unknown dimethyl xylose failed, whereas an authentic specimen of 2:3;—dimethyl xylose prepared from xylan readily yielded a crystalline derivative.

Oxidation of the unknown dimethyl xylose with bromine water yielded a dimethyl δ-xylonolactone which crystallised after standing for several months, m.p. 67°, $[\alpha]_D^{18°}$ +41.2° in water, falling rapidly to an/

an equilibrium value of +31.3° in 6 hours. This product was quite different from a specimen of 2:3-dimethyl γ-xylonolactone (2) prepared in an identical manner for purposes of comparison, $[\alpha]_D^{16°}$ changing from +95° to +68.5° in 500 hours. The hydroxyl group on position C_4 was therefore occupied by a methoxyl residue. The unknown lactone also yielded a syrupy amide (cf. crystalline 2:3-dimethyl xylonamide (4))which gave a positive Weerman reaction indicating the presence of a free hydroxyl groupon position C_2. This together with the properties of the lactone suggested that the xylose derivative was 3:4-dimethyl xylose. Further confirmation of this view was obtained as follows:-

(1) Oxidation of the dimethyl methylxyloside with nitric acid and subsequent esterification led to the formation of an optically active product, the analytical composition of which corresponded closely to that of a hydroxy dimethoxy methylglutarate. In the preparation of the amide of this hydroxy ester a few crystals were obtained m.p. 270°, which were probably a dimethoxy succinamide, but the main product was an uncrystallisable syrup which gave a positive/

positive Weerman test showing the presence of a free hydroxyl group in the α-position. Methylation of this hydroxy dimethoxy xyloglutaric ester with silver oxide and methyl iodide yielded an optically inactive mobile ester the methoxyl content of which corresponded closely with that of i-xylo-trimethoxy methylglutarate. This inactive ester yielded a crystalline amide which proved to be i-xylo-trimethoxy glutaramide. If the original dimethyl xylose had carried methoxyl groups on C_2 and C_4 the hydroxy dimethoxy glutaric ester produced on oxidation would have shown no optical activity.

(2) An osazone was prepared from the free dimethyl sugar without loss of methoxyl groups. This proved that position C_2 was free. It must be admitted however, that the osazone was not crystalline and this result is therefore open to critisism.

It must be concluded therefore that fraction IIa was a mixture of 3:4-dimethyl methylxylosides.

From the properties of fraction III it was thought that this fraction might contain the uronic acid or aldobionic acid portion of the methylated polysaccharide but this proved to be ill-founded since analysis showed that there was only a small amount of an ester grouping present. Complete methylation of/

of fraction III followed by hydrolysis yielded crystalline 2:3:4-trimethyl xylose and on treatment of the mixture of fully methylated sugars with aniline a small amount of a crystalline anilide which proved to be 2:3:4:6-tetramethyl galactose anilide was isolated. From the yields of these two crystalline compounds it appeared that fraction III was composed chiefly of partially methylated xylosides together with a small proportion of methylated galactosides. Hydrolysis of this fraction with 7% hydrochloric acid followed by oxidation of the product with bromine water led to the isolation of a δ-lactone, $[\alpha]_D^{16^\circ}$ in water $+50.1^\circ$ falling to $+40.0^\circ$ in 6 hours. This lactone gave a syrupy amide which gave a positive Weerman test and from these properties it is clearly similar to the 3:4-dimethyl δ-xylonolactone already described. The quantity available was not sufficient for a more detailed study and since the 3:4-dimethyl δ-xylonolactone from fraction IIa did not crystallise until the experimental work was completed a direct comparison was unfortunately impossible. Nevertheless the data suggest that the xylose derivative present in fraction III is mainly 3:4-dimethyl xylose.

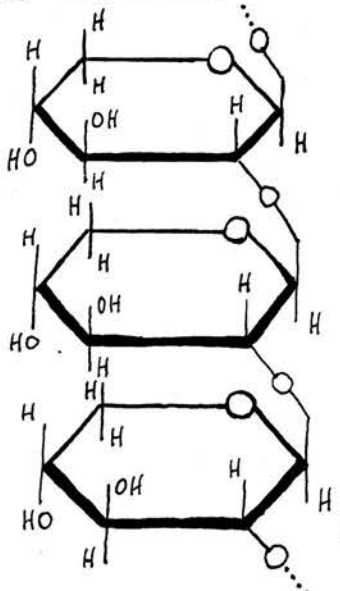
Fraction IV/

Fraction IV has not been thoroughly investigated but a few points have been discovered. A test with barium hydroxide showed the presence of an ester residue. Hydrolysis led to the separation of two fractions, namely a syrup 'A' and a barium salt 'B'. Methylation of 'A' followed by hydrolysis and anilide formation yielded a small amount of 2:3:4:6-tetra-methyl galactose anilide showing the presence of a galactose residue as in fraction III. The yield of anilide was small indicating that the bulk of the syrup was not a galactose derivative. Unfortunately time did not permit of furtherstudy of this portion. On treatment with aniline 'A' itself yielded a crystalline anilide in poor yield, apparently identical with 2:4:6-trimethyl galactose anilide. If all the galactose is present as 2:4:6-trimethyl galactose it follows that pyranose units are again concerned but in this case linked by 1:3-linkages as in agar (9). The barium salt 'B' has not been identified. It was suspected to be the salt of a dimethyl galacturonic acid (p.46) but complete methylation followed by hydrolysis and oxidation has failed to yield crystalline 2:3:4-trimethyl methyl mucate, a syrupy product only being obtained. The fact that on contact with 1% methyl-alcoholic hydrogen/

hydrogen chloride at room temperature the specific rotation of the barium salt did not become negative (p.51), showing that either it was not a derivative of galacturonic acid or that if so the hydroxyl group on carbon atom 4 was occupied by a methoxyl residue.

From the non-reducing character of the polysaccharide and from the strongly negative rotations of the acetylated and the methylated polysaccharide it would seem that there is a preponderance of β-linkages in the molecule, since it is composed chiefly of d-xylose, and carbon atom 1 must be concerned with the linkages in every case. Fraction IIa has been shown to be a mixture of 3:4-dimethyl methyl-xylosides so that it appears probable that the xylose units are linked by 1:2 β-linkages and in addition it follows that they must be xylopyranose units.

The following diagram gives an idea of a possible arrangement provided that one assumes these anhydroxylose units to be contiguous although this is not necessarily the case.



Until more is known about the composition of the other fractions it is obvious that this suggestion must be regarded as tentative but since xylose is the main constituent of the polysaccharide it seems not improbable that some of the anhydroxylopyranose units are united in this way probably terminated by a xylopyranose "end group".

Summary.

1. Direct methylation of the polysaccharide proved unsuccessful but it was found possible to prepare an acetate which was subjected to simultaneous deacetylation and methylation.

2. Hydrolysis of the methylated polysaccharide yielded four fractions (as glycosides) which on subsequent hydrolysis yielded:-

(a) 2:3:4-trimethyl α-d-xylose.

(b) a syrupy dimethyl xylose

(c) a mixture of the dimethyl xylose and a methylated galactose — apparently 2:4:6-trimethyl galactose as shown by the isolation of tetramethyl galactopyranose anilide (after methylation) and 2:4:6-trimethyl galactose anilide on direct treatment with aniline.

(d) a syrup containing a dimethyl uronic acid and a partly methylated galactose.

3. (c) and (d) were not investigated thoroughly but (b) was shown to be 3:4:-dimethyl xylose as follows:-

(i) Complete methylation and hydrolysis gave crystalline trimethyl xylopyranose.

(ii) Oxidation yielded a crystalline δ-lactone.

(iii) The dimethyl xylonamide prepared from this lactone contained an α-hydroxyl group as determined by the Weerman reaction.

(iv) /

(iv) Oxidation with nitric acid yielded an optically active hydroxy dimethoxy glutaric ester which gave an amide which gave a positive Weerman reaction. Further methylation of this hydroxy ester yielded i-xylo-trimethoxy glutaric ester as shown by its conversion to crystalline i-xylo-trimethoxy glutaramide.

4. It is tentatively suggested on this evidence that the xylose residues (on 70% of the molecule) are united by β-linkages through the hydroxyl groups on C_1 and C_2 although apart from the fact that 2:4:6-trimethyl galactose has been identified in fractions III and IV no precise evidence is available as to the constitution of the rest of the products of the hydrolysis of the methylated mucilage.

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The author wishes to express his debt to the Carnegie Trust for the scholarship which enabled him to carry out the work described in this thesis.

To his Director, Dr. E.G.V. Percival, the author owes inexpressible thanks for the advice, encouragement and help, liberally given throughout the period of research.